

**Motor recovery following ischaemic stroke in
humans: insights from Transcranial Magnetic
Stimulation and imaging**

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PhD Thesis

Declaration

I, Orlando Swayne, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Orlando Swayne

Abstract

Recovery of upper limb function after stroke is associated with reorganisation of cortical motor control, but the mechanisms underlying this process in humans remain unclear. We used Transcranial Magnetic Stimulation (TMS) to probe the natural history of neurophysiological reorganisation acutely and chronically after stroke. We then investigated the use of repetitive TMS as an intervention to interact with learning-associated physiological changes, aiming to enhance the rate at which healthy subjects and patients after stroke learn a novel motor task. Physiological measures acquired longitudinally after stroke revealed an immediate shift from intracortical inhibition towards facilitation in both hemispheres. Correlations of intracortical excitability measures with clinical scores emerged by 3 months, suggesting that disinhibition provides access to remote cortical networks which become clinically relevant during this period. A subsequent experiment used paired coil TMS, and concurrent TMS during functional Magnetic Resonance Imaging, to study cortico-cortical interactions after stroke. The contralesional dorsal premotor cortex showed disinhibition in its interaction with the ipsilesional motor cortex and greater motor state-dependent influence on this region in more impaired patients, suggesting a constructive interhemispheric interaction with the affected hemisphere. In healthy subjects the facilitatory effect of Theta Burst Stimulation (TBS) on cortical excitability was enhanced and prolonged by nicotine, but not by levodopa or dextro-amphetamine. Using a thumb movement task, TBS successfully enhanced subsequent motor learning but this effect was blocked by nicotine. TBS increased motor variability, which correlated with learning, and also increased the directional dispersion of evoked thumb movements. This suggests a constructive role for motor output variability in this training paradigm.

In chronic stroke, either TBS or levodopa accelerated training in a similar task but without improving final performance. Levodopa alone was associated with overnight consolidation. This work supports a potential role for physiological and/or pharmacological interventions as adjuncts to post-stroke therapy.

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Publications and attributions

The following publications arose from the period during which the work of this thesis was performed (chronological order):

Swayne O, Rothwell J, & Rosenkranz K (2006) Transcallosal sensorimotor integration: effects of sensory input on cortical projections to the contralateral hand. Clin Neurophysiol 117:855-863.

Ward NS, Newton JM, Swayne OB, Lee L, Thompson AJ, Greenwood RJ, Rothwell JC, Frackowiak RS (2006) Motor system activation after subcortical stroke depends on corticospinal system integrity. Brain 129(Pt 3):809-19.

Teo JT, Swayne OB, Rothwell JC (2007) Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol 118(7):1649-51.

Ward NS, Newton JM, Swayne OB, Lee L, Thompson AJ, Greenwood RJ, Rothwell JC, Frackowiak RS (2007) The relationship between brain activity and peak grip force is modulated by corticospinal system integrity after subcortical stroke. Eur J Neurosci 25(6):1865-73.

Bestmann S, Swayne OB, Blankenburg F, Ruff CC, Haggard P, Weiskopf N, Josephs O, Driver J, Rothwell JC, Ward NS (2008) Dorsal Premotor Cortex Exerts State-Dependent Causal Influences on Activity in Contralateral Primary Motor and Dorsal Premotor Cortex. Cereb Cortex 18:1281-1291.

Reis J*, Swayne OB*, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, Perez MA, Ragert P, Rothwell JC, Cohen LG (2008) Contribution of transcranial

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Swayne OB, Rothwell JC, Ward NS, Greenwood RJ (2008) Stages of Motor Output Reorganization after Hemispheric Stroke Suggested by Longitudinal Studies of Cortical Physiology. *Cereb Cortex* 18:1909-1922

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Obeso JA, Jahanshahi M, Alvarez L, Macias R, Pedroso I, Wilkinson L, Pavon N, Day B, Pinto S, Rodríguez-Oroz MC, Tejeiro J, Artieda J, Talelli P, Swayne OB, Rodríguez R, Bhatia K, Rodríguez-Díaz M, Lopez G, Guridi J, Rothwell JC (2009) What can man do without basal ganglia motor output? The effect of combined unilateral subthalamotomy and pallidotomy in a patient with Parkinson's disease. *Experimental Neurology* 220(2):283-92.

Swayne OB, Teo JT, Greenwood RJ, Rothwell JC (2009) The facilitatory effects of intermittent theta burst stimulation on corticospinal excitability are enhanced by nicotine. *Clin Neurophysiol* 120(8):1610-5.

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Bestmann S*, Swayne OB*, Blankenburg F, Ruff CC, Teo J, Weiskopf N, Driver J, Rothwell JC, Ward NS (2010) The role of contralesional dorsal premotor cortex after stroke as studied with concurrent TMS-fMRI. *J Neurosci* 30(36):11926-37.

Teo JT*, Swayne OB*, Cheeran B, Greenwood RJ, Rothwell JC (2010) Human Theta Burst Stimulation Enhances Subsequent Motor Learning and Increases Performance Variability. *Cereb Cortex* [Epub ahead of print].

(* denotes joint first author)

Attribution of experimental work

In Chapter 4, all experiments were performed both by the author and by Dr Sven Bestmann. The TMS data was analysed by the author, while analysis of the MRI data was performed by Dr Bestmann. In Chapter 5, Experiment 1 was performed by the author, while Experiment 2 was conceived by the author but performed by Dr James Teo. The trial-by-trial behavioural analysis and subsequent computer modelling was performed by both the author and Dr Teo. Experiment 3 was conceived as an additional arm to this study and was performed by Dr Binith Cheeran. All other experiments presented in the current work were performed and analysed by the author.

Chapter 1

Introduction

In this work we describe experiments using Transcranial Magnetic Stimulation (TMS) to acquire physiological measures probing the natural history of neurophysiological reorganisation after stroke, and in combination with imaging techniques to study cortico-cortical interactions in the chronic state. We also investigate the use of repetitive TMS, in combination with medication, as an intervention to interact with learning-associated physiological changes, aiming to enhance the rate at which healthy subjects and patients after stroke learn a novel motor task. Here we describe the background to this work, and how the techniques used can provide information about the physiology of motor control.

1.1 The human cortical motor output

The pyramidal cells originating in layer V of the human primary motor cortex contribute approximately 40% of the one million or so axons making up the corticospinal tract (Jane et al 1967), and some of them provide direct monosynaptic projections to the alpha motor neurones of the spinal cord. Of these around 90% cross in the medullary decussation, providing a predominantly contralateral projection to the spinal cord. Significant projections are also received from the premotor cortex (Dum & Strick 1991), and from brainstem regions including the red nucleus (rubrospinal projection), lateral vestibular nuclei (vestibulo-spinal projection), the pontine and medullary reticular formation (reticulospinal projection) and the superior colliculus (tectospinal tract) (Schieber 2007). For the fine control of upper limb muscles and in particular the hand, however, the primary corticospinal projection is the most important and the most direct. The primary motor cortex in turn receives numerous projections from the thalamus and from inter-connected cortical regions, including dorsal and ventral premotor cortex, prefrontal cortex, sensory cortex,

posterior parietal cortex and the supplementary motor area. Far from acting as the passive servant of ‘higher’ cortical regions, the primary cortex is believed to perform a complex integration of inputs from these regions, the result of which is motor behaviour. Conveniently located on the surface of the cerebral convexity, the primary motor cortex is readily accessible to Transcranial Magnetic Stimulation, resulting in measurable evoked potentials in hand muscles. Thus assessed, this region provides a real-time window into the physiological interactions taking place within cortical motor networks.

1.2 Transcranial Magnetic Stimulation (TMS)

1.2.1 Induction of a corticospinal volley

During TMS, a tightly-wound copper coil (most commonly in a figure-of-eight formation) is laid with the coil’s plane flat on the scalp of the subject. A brief, rapidly-changing, electrical current within the coil induces a strong and localised magnetic field perpendicular to the brain’s surface: this itself is rapidly changing and in turn induces an electrical field parallel to the cortical surface sufficient to cause axonal depolarisation, leading ultimately to a Motor Evoked Potential (MEP) in the target muscle. Detailed study of the corticofugal discharge in response to a motor cortical stimulus by Amassian et al (1989) revealed multiple components of the MEP. These can be observed either by epidural recordings or by measuring single motor unit recordings with needle electrodes, and consist of a short latency direct wave (D-wave) followed by several longer latency indirect waves (I-waves). The D-wave is thought to result from direct depolarisation of the initial axon segment of the corticospinal neuron and is most effectively activated in human subjects by transcranial electrical stimulation or high intensity TMS. The I-waves following the

D-wave occur sequentially with a periodicity of approximately 1.5 ms, reflecting the delay required for synaptic discharge. Thus, the first I-wave (I1) is thought to be generated through the depolarisation of an axon synapsing directly onto a corticospinal neuron (i.e. monosynaptically), while following I-waves (I2 and later) may require local polysynaptic circuits. I-waves can be elicited using relatively low TMS intensities in humans and are thus readily amenable to study.

1.2.2 Using TMS to assess corticospinal excitability

TMS can be used to quantify 2 aspects of corticospinal excitability: motor thresholds and motor recruitment curves. Measuring motor thresholds may be done with the muscle entirely at rest or with a degree of pre-activation: resting or active thresholds respectively. The technique employed to measure these parameters is described in Chapter 2. The stimulus intensity required to evoke an MEP depends on 3 main factors: i) axonal excitability in the motor cortex; ii) synaptic excitability onto pyramidal cells within the motor cortex; iii) synaptic excitability onto motoneurons within the spinal cord. Axonal excitability is relatively stable but may be modulated to an extent by recent activity (Vagg et al 1998) and in patient studies is likely to be affected by medications (Ziemann et al 1996a). Synaptic excitability within the cortex and the spinal cord is likely to be susceptible to a number of factors, from pre-activation by motor activity to general levels of arousal. While the above factors give rise to considerable intra-subject variability, a significant degree of inter-subject variability is also widely recognised. These considerations complicate the interpretation of motor thresholds as reliable measures of corticospinal excitability.

Motor recruitment curves are generated by measuring MEP amplitudes (or areas) across a range of stimulus intensities. This gives rise in healthy subjects to a sigmoidal curve whose plateau reflects the maximum output that can be generated from the stimulated region, and whose gradient reflects the rate at which the corticospinal tract is recruited (Ridding & Rothwell 1997). It cannot be assumed that there exists a linear relationship between the number of intact fibres in the corticospinal tract and the recruitment curve plateau, as the degree of depolarisation of motoneurons within the spinal cord will reflect also the number and strength of synaptic contacts and the number of repetitive discharges resulting from a cortical stimulus, both of which may vary across the neuronal population. If using this measure to assess corticospinal tract damage after stroke, the situation may be further complicated by changes in the pattern of I-wave discharges within the motor cortex and perhaps by the appearance of new synaptic connections within the spinal cord (Talelli et al 2006). The recruitment curve gradient reflects the distribution of excitability within the population, and is steeper in active than relaxed muscle.

1.2.3 Using TMS to assess intracortical excitability

Facilitatory and inhibitory interactions occurring locally within M1 can be studied by delivering two TMS pulses through the same coil (or two overlapping coils targeting the same cortical area), referred to generically as paired-pulse TMS (Kujirai et al 1993). Intracortical facilitation (ICF) of a test MEP can be elicited at interstimulus intervals (ISIs) of 6–25 ms, using a subthreshold conditioning stimulus (CS) to influence the response to a subsequent suprathreshold test stimulus (TS). The range of CS intensities at which this occurs differs from that inducing the more robust phenomenon of intracortical inhibition (see below), suggesting that facilitation is not

simply a rebound phenomenon from inhibitions at shorter ISIs (Ziemann et al 1996b). Studies involving patients with cervical extradural electrodes have demonstrated that facilitation observed at 25 ms is mediated within the cortex, but more recent studies have suggested a possible contribution of changes in spinal excitability at shorter ISIs of 10-15 ms (Nakamura et al 1997; Di Lazzaro et al 2006). Excitatory glutamatergic interneurons within M1 and N-methyl-d-aspartate (NMDA) receptors appear to influence ICF (Ziemann 2003). NMDA antagonists have been shown in two separate studies to abolish (dextromethorphan) or even reverse (memantine) ICF measured at 10 or 15ms (Ziemann et al 1998; Schwenkreis et al 1999), suggesting that it is mediated by glutamatergic neurons. ICF is also thought to be modulated by GABA_A activity, since it is reduced by the GABA_A agonist lorazepam and abolished by ethanol, which potentiates GABA-mediated currents (Ziemann et al 1995, 1996a, 2004). This is consistent with the idea that the inhibition of I3 waves that is responsible for short interval inhibition (SICI – see below) may persist as late as 20 ms after the CS (Hanajima et al 1998). Thus the phenomenon of ICF is likely to be influenced by glutamatergic facilitation tempered by persisting GABAergic inhibition.

Two principal types of local intracortical inhibition can be studied using paired pulse TMS. Short interval intracortical inhibition (SICI) was first described by Kujirai et al (1993) and can be elicited by a subthreshold CS followed by supra threshold TS. At interstimulus intervals (ISIs) of 1–6 ms the test motor response is inhibited by the conditioning shock. Two main phases of inhibition have been described, at ISIs of 1 ms and 2.5 ms (Fisher et al 2002; Roshan et al 2003). Two studies have used direct recordings of descending spinal cord volleys to confirm that the initial I1-wave is

suppressed by the CS, indicating that SICI seems to be mediated at the cortical level (Nakamura et al 1997; Di Lazzaro et al 1998). Inhibition of the descending spinal volleys is most pronounced at an ISI of 1 ms and disappears by 5 ms. It has been shown that GABA_A agonists enhance SICI (Ziemann et al 1996a; Ilic et al 2002). However, a single dose of the GABA_A antagonist flumazenil did not alter SICI, suggesting that there might be no tonic activity at the benzodiazepine binding site of the GABA_A receptor in the normal human M1 (Jung et al 2004). It has also become apparent that inhibition at the short ISI of 1 ms does not depend on GABA_A, while ‘true’ SICI at an ISI of 2.5 ms is likely to be mediated by GABAergic inhibition at the intracortical level (Fisher et al 2002; Roshan et al 2003), supporting the point of view that they are mediated by different mechanisms. It is proposed that SICI at an ISI of 1ms may be due to refractoriness or changes in axonal excitability of excitatory interneurons.

Long interval intracortical inhibition (LICI) is elicited by a suprathreshold CS and TS applied at ISIs of approximately 50–200 ms (Valls-Sole et al 1992; Wassermann et al 1996) – thus two MEPs are elicited, of which the second is smaller in amplitude. Previous evidence has suggested that LICI at ISIs longer than 50ms is mediated within M1 rather than subcortical structures (Nakamura et al 1997). Although this evidence supports the view that LICI is related to reduced cortico-fugal excitability, it still remains unclear whether the same population of neurons mediates LICI and SICI. Pharmacological studies suggest that LICI is mediated by GABA_B receptors (Werhahn et al 1999; McDonnell et al 2006) while SICI is primarily mediated by GABA_A receptors (Ziemann 2003). Nevertheless, the involvement of different

receptor subtypes does not in itself exclude the possibility of a shared neuronal population mediating these two inhibitory phenomena.

1.2.4 Using TMS to assess cortico-cortical interactions

A paired pulse approach can also be applied to study interactions between remote but anatomically connected cortical regions, with the conditioning and test stimuli delivered through 2 separate TMS coils. A great deal of work over the past decade has gone into characterising interactions between a number of cortical regions and the primary motor cortex, in addition to cerebello-cortical and sensorimotor interactions: these are summarised in Reis et al 2008. These interactions are summarised schematically in Figure 1.1.

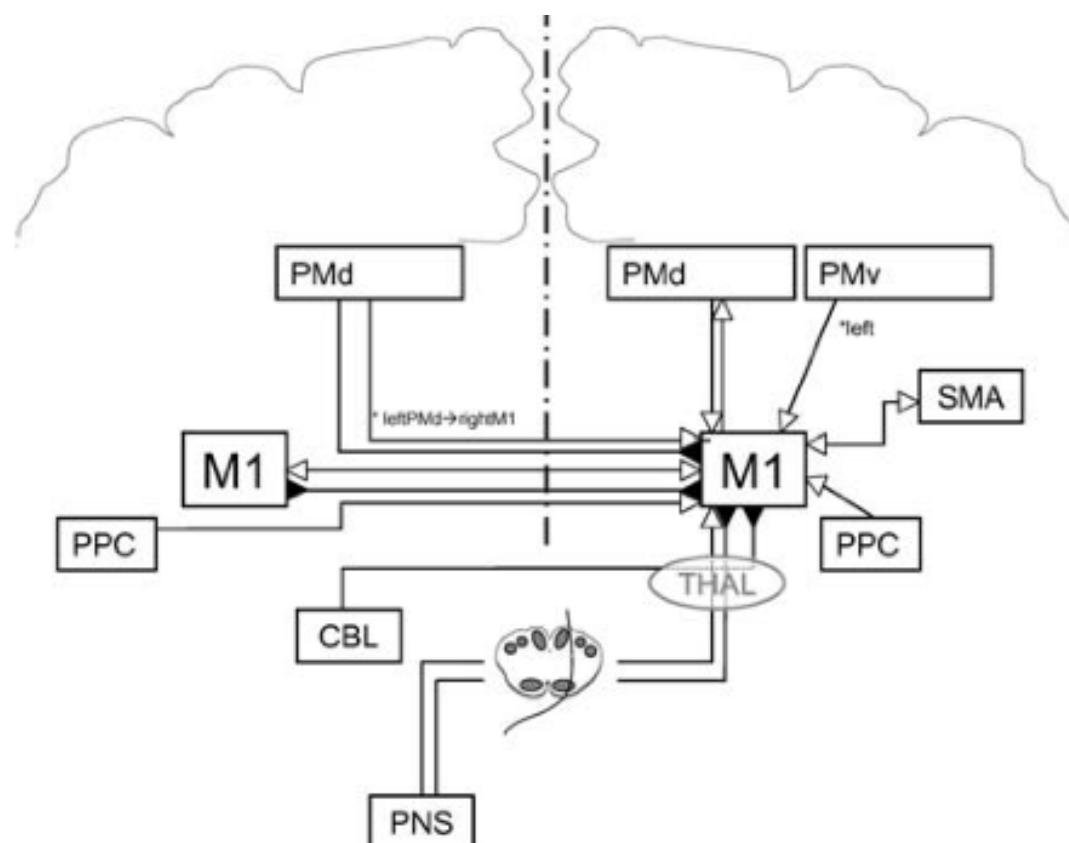


Figure 1.1 Summary of inter-regional influences on the primary motor cortex

The currently described influences of other brain areas on the output of the primary motor cortex (M1) are shown. Open arrows denote facilitation, while filled arrows denote inhibition. In many cases the influence shown represents a net effect of several specific interactions. These influences include projections from motor areas in the ipsi- and contralateral hemispheres and the effects of afferent sensory input. From Reis et al 2008. PMd = dorsal premotor cortex; PMv = ventral premotor cortex; SMA = supplementary motor area; PPC = posterior parietal cortex; CBL = cerebellum; THAL = thalamus; PNS = peripheral nervous system

1.2.4.1 Interhemispheric interactions between the motor cortices

Transcallosal projections between the two M1 hand areas are known to exist in monkeys (Jenny 1979). That such projections can convey information between the hemispheres is suggested by the detection of evoked potentials over M1 following electrical or magnetic stimulation of the contralateral M1, both in animal models and in humans (Hanajima et al 2001; Chowdhury & Matsunami 2002). Using a paired pulse TMS technique with one coil over each M1 hand area, Ferbert et al (1992) investigated interactions between the two M1s. While inhibition was their most striking finding (see below), they also described a facilitation occurring in some subjects, at shorter ISIs, which was ‘capricious’ and poorly reproducible. This phenomenon was further investigated by Hanajima et al (2001), who found that such interhemispheric facilitation (IHF) is reliably obtainable under particular conditions: small test MEP, slight voluntary pre-contraction of the target muscle, antero-posterior test stimulus and low intensity conditioning stimulus. Baumer et al (2006) demonstrated reliable IHF at rest following a conditioning stimulus to M1 at two very subthreshold intensities. At 60% of active motor threshold, IHF occurred at an interval of 6 ms, with the TS current in a postero-anterior (PA) direction (unlike

Hanajima et al). At 80% of AMT, IHF occurred at 6–8 ms ISI, with the TS current in an antero-posterior (AP) direction. The I-wave components of the test pulse affected in these two conditions are likely to be predominantly I1 and I3, respectively. The authors suggested that the longer ISIs could be explained by the activation of slower-conducting fibres at these lower CS intensities, and that at higher intensities such facilitation may have been overwhelmed by concomitant inhibition. In cats the cortical area of the distal forelimb has an excitatory transcallosal connection to the homologous motor cortex, but this is surrounded by a larger area of inhibition (Sanuma & Okuda 1962). It may be that the relatively poor spatial resolution of TMS means that this robust surround inhibition predominates in most circumstances.

In contrast to interhemispheric facilitation, interhemispheric inhibition (IHI) is more robust and occurs over a wide range of ISIs (6–50ms) (Ferbart et al 1992; Daskalakis et al 2002). This form of inhibition is lacking in patients with ischaemic lesions affecting transcallosal populations, supporting the idea that this phenomenon is mediated via the corpus callosum (Boroojerdi et al 1996). Emerging evidence suggests that IHI elicited at relatively short ISIs (e.g. 8–10ms) is mediated by different mechanisms than that elicited at longer intervals (e.g. 40ms). Other than the ISI, the stimulation parameters required to elicit IHI10 and IHI40 are similar. Both require a suprathreshold CS and TS intensity adequate to elicit an MEP of 0.5–1.5 mV in amplitude (Kukaswadia et al. 2005). Both are also believed to be dependent on GABA_B-mediated neurotransmission in the target hemisphere (Daskalakis et al 2002; Kukaswadia et al 2005). This was confirmed for long latency IHI by a recent study of pharmacological modulation by GABA_A agonists: IHI at ISIs of up to 200 ms was strengthened after application of the GABA_B agonist baclofen, suggesting that

long interval IHI is most likely mediated by postsynaptic GABA_B receptors (Irlbacher et al 2007). Evidence for differing mechanisms mediating IHI at these 2 intervals comes from studies investigating the interactions between IHI and other inhibitory phenomena, both within the conditioning and target hemispheres: details of these investigations are beyond the scope of this chapter.

The approach of paired pulse TMS between the two M1 hand areas has thus revealed at least three facilitatory and two inhibitory distinct interactions, depending on the parameters used (ISI, coil orientation and intensities of CS and TS). Facilitation or inhibition can even be produced at overlapping ISIs, depending on the nature of the CS and TS, suggesting that such interactions are likely to occur in parallel. With regard to the cell populations involved, it is likely that even in the case of IHI the transcallosal projections are excitatory, synapsing onto local inhibitory circuits within the target hemisphere. It is not at present possible to infer whether the various phenomena are mediated by distinct transcallosal populations or whether common projections are used with, for example, different coding characteristics.

1.2.4.2 Premotor cortical influences on the primary motor cortex

The dorsal premotor cortex (PMd) has attracted particular attention in regard to its influence on the primary motor cortex because of its recognised role in movement selection (Cisek & Kalaska 2005) and its dense anatomical connection to M1 in monkeys (Ghosh & Porter 1988a). Two main approaches have been taken to investigating PMd's influence on its ipsilateral M1. The first involves applying repetitive TMS (rTMS) to PMd, using a protocol known to up- or down-regulate cortical excitability, and afterwards assessing motor cortical excitability in M1 with

single pulse TMS. An rTMS protocol used to produce a transient reduction in excitability, applying subthreshold stimuli at 1Hz, was applied to PMd and resulted in a reduction of MEP amplitudes elicited from M1 (Gerschlagel et al 2001) but increased paired pulse excitability at a 7 ms ISI and shortened the cortical silent period (Munchau et al 2002). Conversely, applying an rTMS protocol that increases cortical activity (5 Hz at 90% AMT) to PMd had the opposite effects: MEP amplitudes were increased and paired pulse excitability at 7 ms ISI was reduced (Rizzo et al 2004). Together, these rTMS studies show that manipulations of PMd excitability modulate M1 corticospinal excitability in a similar direction, suggesting at first glance a facilitatory influence of PMd on M1.

The second approach has employed two coils in a paired pulse protocol. Civardi et al (2001) showed that a subthreshold CS (defined by the M1 MEP threshold) over PMd reduces the excitability of the ipsilateral M1, with a maximum effect at an ISI of 6ms – this ipsilateral PMd–M1 inhibition requires a CS given at 90% of AMT with antero-posterior current flow. The authors argued that this interaction did indeed involve conditioning PMd rather than acting via current spread to M1, on the basis of spatial separation (conditioning at an intermediate point produces no inhibition), temporal separation (a time course that is distinct from SICI) and the effect of coil orientation (Civardi et al 2001). However, in addition to this inhibitory interaction, facilitation could also be elicited if a higher conditioning intensity was used (120% AMT). Mochizuki et al (2004) applied a similar paired pulse approach to investigate the interhemispheric interaction between PMd and the contralateral M1. At ISIs between 4 and 20ms (with a CS to the right PMd and a TS to the left M1), they found significant inhibition of the test MEP using a CS intensity of either 90% RMT (with

an ISI of 8ms) or 110% RMT (ISI of 8–10ms). This interhemispheric PMd–M1 inhibition is spatially specific for PMd (as not detected when stimulating 2 cm anterior, lateral or medial to the target area) but not for the hemisphere (Baumer et al 2006). Stimulation of the left PMd and right M1 revealed the same results (Baumer et al 2006; Koch et al 2006). This interaction can be distinguished from M1-to-M1 IHI at the 90% CS intensity (on the basis of a lower threshold and differing effects of voluntary contraction) but this distinction is less clear at the suprathreshold intensity. Interhemispheric PMd–M1 inhibition has also been described at the longer interstimulus interval of 150 ms, using a latero-medial CS at 110% of AMT (Mochizuki et al 2004), but at such a long interval the effect cannot be assumed to be transmitted transcallosally. The effect of PMd stimulation on the contralateral M1 seems to depend on the stimulation intensities used, as demonstrated recently by Baumer et al. (2006). Conditioning the left PMd with low stimulus intensity (80% of AMT) and targeting the right M1 (with small test MEPs), interhemispheric PMd–M1 facilitation was described at an ISI of 8 ms (Baumer et al 2006). This facilitation was dependent on a postero-anterior current flow for the TS, providing indirect evidence that this form of facilitation preferentially affects I1 waves in the target hemisphere. A mechanism proposed for these inter-regional effects is the activation of long distance projections from the PMd to ipsi- or contralateral M1, consistent with anatomical studies showing dense connections between those areas, which are known to be both inhibitory and facilitatory (Ghosh & Porter 1988b; Tokuno & Nambu 2000). Details of how these long-range projections interact with the intracortical circuits described above are not well known. The only study to directly address this question has been Mochizuki et al (2004), who showed that interhemispheric PMd–

M1 inhibition was associated with a reduction in intracortical inhibition in the target hemisphere (SICI at ISI of 2 ms).

The idea that cortico-cortical interactions between the premotor and primary motor cortex may involve modulation of corticofugal I-waves receives support from the animal literature. The F5 cortical region in the monkey (equivalent to the ventral premotor cortex in man) not only contributes to the corticospinal projection (Dum & Strick 1991) but also projects to the primary motor cortex (Ghosh et al 1987). Under light sedation Cerri and colleagues demonstrated that the EMG response to direct motor cortical stimulation could be robustly facilitated by a preceding short latency conditioning stimulus delivered to F5 (Cerri et al 2003). Direct recording of corticospinal volleys in the cervical cord has revealed that this protocol induces specific facilitation of the later I-waves (Shimazu et al 2004), while experiments in awake monkeys suggest that such premotor-motor interactions may instead be inhibitory at longer latencies and are specific to the grasping task being performed (Prabhu et al 2009). Modulation of the corticospinal output via changes in later I-waves is consistent with what is known of intracortical circuits in humans as probed by TMS, described above in section 1.2.3.

1.3 The motor consequences of stroke and subsequent recovery

1.3.1 Components of motor recovery

Stroke gives rise to an enormous social and economic burden, likely to become more significant with time in the developed world as a result of the aging population.

Despite advances in the delivery of hyper-acute stroke care, stroke remains the single greatest cause of adult disability (National Audit Office 2005), accounting in the

United Kingdom for 4-6% of the total National Health Service Budget. Persistent motor weakness is largely responsible for the resulting functional impairment, with around 65% of patients unable to incorporate their paretic hand into everyday use at 6 months (Kwakkel et al 2003). Significant spontaneous improvement in motor function is, however, observed over the first weeks and months in a majority of cases (Kwakkel et al 2003). In the acute period this improvement is likely to result from several factors: resolution of local cerebral oedema and of the inflammatory cytokine response, normalisation of systemic metabolic disturbance and treatment of medical co-morbidities such as sepsis and cardiovascular complications. A further contributing factor to early improvements may be the resolution of diaschisis. This term (von Monakow 1914) refers to the effects of dysfunction in structurally intact brain regions, remote from but functionally connected to the site of injury. Such a phenomenon has been observed in animal models of stroke as hypometabolism in remote regions (Carmichael et al 2004), although its contribution in humans is less clear (Cramer et al 2008).

Once into the chronic phase, it is now well established in animal models that axonal and dendritic sprouting result in structural changes both in the peri-infarct region and in connected regions (Carmichael et al 2001; Dancause et al 2005). More recently, new neurons have been observed to appear following stroke as a result of the process of neurogenesis (Ohab & Carmichael 2008). The role of these newly-recognised phenomena in functional recovery is as yet unclear. It should also be emphasised that in the chronic phase functional compensation as a result of behavioural adaptation and environmental modification is likely to play a major part in functional gains. Between the acute and chronic phases, however, it is believed that significant changes in the

organisation of cortical motor networks occur and that this process plays an important role in motor recovery. Evidence for such reorganisation comes from animal models of stroke, and from both functional imaging and physiological studies in humans.

1.3.2 Evidence for reorganisation: animal models

Somatotopic motor representations can be readily studied in animals and over 60 years ago it was observed that recovery of movement after experimental lesions was associated with re-modelling (Glees & Cole 1950). More recently, intracortical microstimulation techniques have demonstrated in squirrel monkeys that small motor cortical lesions resulted in the widespread reduction in excitability of spared adjacent hand representations, which are replaced by adjacent proximal representations (Nudo & Milliken 1996). Similar experiments in un-injured squirrel monkeys have also shown that equivalent somatotopic cortical re-organisation can be induced by motor training (Nudo et al 1996a). The idea that motor training following a cortical lesion has the potential to influence subsequent re-organisation is supported by the retention of the spared cortical hand representation in monkeys who received training but not in the un-trained group (Nudo et al 1996b). Somatotopic changes are also observed in reciprocally connected non-primary motor areas, most notably the ventral premotor (Frost et al 2003; Dancause et al 2005) and supplementary motor areas (Eisner-Jancowicz et al 2008) although the functional significance of such changes is unclear.

Studies of synaptic efficacy suggest that the cortical re-organisation occurring after stroke is underpinned by synaptic plasticity within the horizontal intracortical connections which are thought to define motor map characteristics (Ghosh & Porter 1988a; Cheney et al 1985). Experimentally-induced Long Term Potentiation (LTP)

causes synaptic strengthening but also the expansion of motor maps (Monfils & Teskey 2004). Conversely, changes in the LTP characteristics of motor cortical synapses can be induced by learning new skills (Rioult-Pedotti et al 1998). It has also been observed that synaptogenesis induced by motor learning co-localises to changes in motor maps (Kleim et al 2002). It thus appears likely that the extensive re-organisation revealed by cortical mapping studies in animal models of stroke is dependent upon changes in synaptic efficacy.

1.3.3 Evidence for reorganisation: functional imaging studies in humans

Task-related changes in cerebral blood flow can be studied in healthy humans using imaging modalities that respond to local changes in metabolic activity or blood flow. Positron Emission Tomography (PET) and functional Magnetic Resonance Imaging (fMRI) are the most widely applied techniques with which investigators have attempted to characterise changes in the organisation of motor control after stroke. Early PET studies compared the patterns of activation observed when moving the paretic (or normal) hand in patients with subcortical infarcts versus healthy groups, and demonstrated greater task-related activation in premotor cortex (dorsal and ventral), supplementary motor area and cingulate cortex bilaterally (Chollet et al 1991; Weiller et al 1992; Calautti et al 2001). The use of fMRI to measure blood oxygen level-dependent signal (BOLD signal) has produced similar results (Cramer et al 1997). This approach has also demonstrated shifts in motor representations, variously reported to occur in a ventral (Weiller et al 1992) or caudal (Pineiro et al 2001) direction.

A subsequent cross-sectional study tested hand movements in a group of patients with a range of motor impairment, using a hand grip task that did not depend upon the ability to perform fractionated finger movements. Negative correlations were observed between task-related activation and clinical scores of motor function in a number of cortical regions, including the main motor network described above (Ward et al 2003). A follow-up study (including the author – not described in Results chapters) related task-related blood flow to a TMS measure of corticospinal tract excitability, demonstrating a similar negative relationship across the cortical motor network (Ward et al 2006). Thus it appears that in response to injury there is progressive recruitment of non-primary and contralesional motor areas with increasing disruption of the primary corticospinal motor projection. In a longitudinal study, a group of patients with ultimately good recovery performed the same hand grip task on an average of 8 occasions after stroke. The initial pattern of over-activation across this network gradually evolved with recovery into a more lateralised and ‘physiological’ motor activation pattern (Ward et al 2003b), although it is recognised that bilateral motor activation persists in some patients (Feydy et al 2002).

The way in which the non-primary and contralesional motor regions interact with the original motor system, and the role which they play in hand movement, is not clear at present. Although the premotor cortex and supplementary motor area contribute fibres to the corticofugal projection to the spinal cord (Dum & Strick 1991; Strick 1988) they are less efficient in exciting spinal neurons than the primary projection (Boudrias et al 2006). Moreover, there is some evidence from both fMRI and TMS studies to suggest that contralesional activation during movement may exert a negative influence on movement of the paretic hand, perhaps via excessive interhemispheric

inhibition (Grefkes et al 2008; Murase et al 2004). This is supported by therapeutic studies which have aimed to improve paretic hand movement by reducing activity in the contralesional primary motor cortex (see below). However there is also reason to believe that some non-primary contralesional motor regions may play a positive role in recovered motor function. Experiments in which brain activity is transiently disrupted using TMS have demonstrated that motor responses can be interrupted in this way in patients with chronic stroke when applied to the dorsal premotor (PMd) cortices of the ipsi- and contra-lesional hemispheres, but not in healthy subjects (Johansen-Berg et al 2002; Lotze et al 2002; Fridman et al 2004). In particular, movement was degraded more in ipsilesional PMd in patients with better recovery and contralesional PMd in patients with poor recovery, suggesting functional recruitment of the contralesional PMd in the face of more extensive injury. In an fMRI study of patients with chronic stroke (including the author – not described in Results chapters) we tested specifically for regions in which there was a linear relationship between force production and BOLD signal, an ‘executive’ property observed usually in the primary motor cortex. In patients with more corticospinal tract disruption this property was present to a greater degree in bilateral ventral premotor, contralesional PMd and contralesional primary motor cortex but not in ipsilesional primary motor cortex (Ward et al 2007). This suggests that an executive role may be adopted by contralesional and non-primary motor regions in response to injury, supporting a functional role in recovered movement.

1.3.4 Evidence for reorganisation: TMS studies in humans

The advent of TMS has made it possible to obtain in vivo physiological data from the motor cortices of patients at a range of stages after stroke, and has produced a number

of insights into the pathophysiological changes occurring. Abnormalities of corticospinal excitability have primarily been used in attempts to quantify the damage to the corticospinal projection (1.3.4.1); parameters reflecting intracortical excitability have been used to explore mechanisms of recovery (1.3.4.2); abnormal interhemispheric interactions have been used to support the notion of excessive inhibition of the affected hemisphere by the unaffected hemisphere (1.3.4.3).

1.3.4.1 Abnormalities of corticospinal excitability

A single TMS pulse to the cortex actually results in repetitive discharge of corticospinal neurones at high frequency (600Hz: I-waves) due to reverberation of activity in intracortical circuits. Receipt of 2 or more of these descending volleys brings resting spinal motoneurones to threshold. Given this sequence of events, it is evident that MEPs ought to be able to provide some estimate of the functional integrity of the corticospinal tract after stroke (Talelli et al 2006). However it should also be clear that since MEPs rely on a rather complex sequence of events, involving not only corticospinal conduction but also synaptic transmission at cortex and cord, the interpretation of changes can sometimes be complex.

Two main measures have been used to quantify corticospinal function after stroke: (a) the threshold (MT) for generating an MEP response and (b) the relationship between the intensity of the TMS at suprathreshold levels and the amplitude of the evoked MEP. When the TMS intensity is gradually increased, in steps commonly expressed as percentage of the MT, an input-output (I/O) curve can be generated. In practice, many researchers have measured the MEP amplitude at a single point of the I/O curve. The threshold depends on the excitability of cortical axons and synapses

(Ziemann et al 1996a), whereas the slope of the I/O curve depends on the distribution of excitability in the corticospinal projection as well as the total number of available fibres in that connection (Devanne et al 1997).

Three main factors are responsible for the changes in threshold and the contribution of each will depend on the lesion location and load. (1) Changes in the ionic composition of extracellular fluid. At a cortical level these can increase the threshold for activating axons; in capsular strokes they may reduce or block conduction in corticospinal axons. These effects should resolve relatively quickly after the stroke (Furlan et al 1996). (2) Altered excitability of synaptic connections at both cortex and spinal cord. In the cortex, there may be disconnection from peripheral afferent inputs in the case of a subcortical lesion, or corticocortical inputs in the case of pure cortical strokes. In both cases, this will affect the excitability of post-synaptic neurones and increase thresholds. At the spinal cord, loss of any tonic descending facilitation will also reduce spinal motoneuron excitability and again increase thresholds. (3) Related to both these considerations is the fact that multiple descending volleys are necessary to activate spinal motoneurons especially with the target muscle at rest. The system may fail to produce these because of changes in the excitability of intracortical circuits or in the membrane properties of corticospinal neurones. In addition any compromise of axonal conduction in the internal capsule may cause conduction block of repetitive transmission to the cord.

Similarly, the amplitude of the MEP may be reduced for a number of reasons. One possibility is that there are not enough working connections available to a standard suprathereshold TMS pulse. Indeed, failure to produce repetitive firing and

dysynchronisation of the descending impulses at spinal level could also result in smaller multiphasic MEPs commonly seen in stroke patients. In theory, however, the same could be seen if the remaining connections were adequate in numbers but the distribution of excitability was skewed towards higher values. In this situation, threshold measurements could even remain relatively normal but typical increments in stimulation intensity might not be enough to recruit additional fibres. In this case the slope of the I/O curve would be reduced. If the stimulator's output was enough to activate all the available connections, the plateau of the curve, i.e. the maximum available output, should not be affected. On the contrary, a critical reduction in the number of fibres would additionally affect the plateau level. Obviously, the plateau level is a major determinant of the gradient of the curve, thus such interpretations are really informative when a plateau has been reached. Finally, as with threshold assessments, I/O curves are subject to excitability changes in the spinal cord. In most instances, active and resting measures, mainly in terms of threshold and MEP amplitude, show similar trends which suggests that the cause of the abnormalities cannot be placed solely at spinal level (Catano et al 1995; Cicinelli et al 1997; Traversa et al 1998). Additional support comes from studying spinal reflex arcs, such as H-reflexes and F-waves, which do not appear to be changed, at least within the first few months after the stroke (Manganotti et al 2002; Traversa et al 2000).

Given the complexity of the events following a TMS pulse it is not surprising that the results reported in the literature have been relatively variable. As a general rule, TMS often fails to elicit responses in the affected hand muscles (Catano et al 1995; Manganotti et al 2002; Trompetto et al 2000; Delvaux et al 2003). When responses are present, increased threshold and reduced MEP amplitudes can be expected. In most

instances measures improve with time after stroke, tending to reach a plateau after about 3-6 months, paralleling the usual time course of improvement of motor symptoms (Cicinelli et al 1997; Traversa et al 1998; Catano et al 1996). Often this improvement is incomplete and abnormal TMS values persist in the chronic stage even when clinical recovery is good (Byrnes et al 2001; Thickbroom et al 2002).

Most change in TMS measures of corticospinal excitability is usually observed within the first 90 days. Thresholds appear to be the first measure to reach a plateau (Traversa et al 2000), which sometimes can lie within the normal range, but the evolution of excitability measures with time is unclear. It appears commoner for MEP amplitudes to remain abnormal in the long-term, and it is possible that the I/O relationship is more difficult to normalise, depending both on the distribution of excitability and on the availability of corticospinal connections.

There is some uncertainty regarding the relationship between these TMS measures and clinical recovery. Early clinical improvement is most probably related largely to reperfusion of the ischaemic penumbra and resolution of oedema resulting in reinstitution of connections that have been malfunctioning but not critically damaged (Furlan et al 1996). These events could also underlie the electrophysiological improvement seen within the first few weeks, at which point the lesion load should be final. Indeed, many authors suggest that during this stage both thresholds (Catano et al 1996) and amplitude of the MEP (Traversa et al 2000) show some association with severity of symptoms. However it is not clear whether this relationship persists with time.

1.3.4.2 Abnormalities of motor maps and intracortical excitability

As discussed above, animal models suggest that changes occur in cortical motor maps – the areas of cortex from which movements may be evoked – in the regions surrounding but not directly involved in an area of cortical infarction. It seems reasonable that such a process may prove helpful to recovery, either by recruiting adjacent intact cortex (in the case of a cortical lesion) or by providing access to an intact corticospinal outflow tract (in the case of a subcortical lesion). In support of this, cross-sectional and longitudinal TMS mapping studies in conscious stroke patients comparing the motor hand representations of the affected and unaffected hemispheres have shown that the ‘centre of gravity’ of such representations may shift in the ipsilesional side often by several centimetres (Delvaux et al 2003; Bastings et al 2002). These shifts are not usually present early after the stroke, suggesting that they may occur by means of a gradual cortical process similar to that seen in primates (Delvaux et al 2003; Byrnes et al 2001; Thickbroom et al 2002; Bastings et al 2002). Some have suggested that greatest shifts tend to be seen after dense subcortical strokes which disconnect a significant area of cortex (Cicinelli et al 1997). Although results have been variable, a positive correlation has been reported between the magnitude of map shift and motor recovery in a group of patients with intact corticospinal excitability (Thickbroom et al 2004), suggesting that such a phenomenon may represent a constructive adaptive response to injury.

How do such changes in motor representations occur? Animal studies have pointed to the importance of horizontal cortical connecting fibres as potential candidates. There is evidence that cortical reorganisation depends on the removal of GABAergic

intracortical inhibition, being enhanced or blocked by GABA antagonists or agonists respectively. A form of intracortical inhibition (short interval intracortical inhibition, SICI) which is GABA-dependent can readily be measured using paired pulse TMS, and is known to be reduced in the context of normal motor learning, which is widely used as an analogy for recovery following stroke (Liepert et al 1998). A number of studies have therefore investigated whether such a release from GABAergic inhibition may play a role in allowing the reorganisation of motor representations after stroke.

Reduced SICI in the affected hemisphere has been widely reported in the acute period after stroke (Cicinelli et al 2003; Manganotti et al 2002; Liepert et al 2000a) and in some investigations in the chronic stage (Shimizu et al 2002). A second form of GABAergic inhibition, termed long interval intracortical inhibition (LICI), is as yet untested after stroke, while intracortical facilitation (a possible glutamatergic phenomenon) has been consistently reported as being normal. The presence of clear disinhibition would be consistent with reduced GABAergic activity and would favour reorganisation according to animal models. However, without more invasive tests, it is not possible to be certain whether this represents a constructive 'response' to injury or an epiphenomenon. Finally, when interpreting the results of paired pulse TMS experiments it is worth bearing in mind that while certain parameters (SICI, LICI etc) are commonly ascribed to specific intracortical populations the reality may be more complex. The investigator can simply measure the effect of the conditioning pulse on the test pulse, which may reflect the overlapping influences of several neuronal populations. Recent studies in healthy subjects have begun to tease out these contributions by using a variety of intensities and orientations of conditioning and test pulses, but these have yet to be applied to patient groups.

There has been considerable interest in studying physiological changes in cortical motor areas in the ‘intact’ contralesional hemisphere. This has stemmed from the finding of altered contralesional cortical excitability in animal models of stroke, and also from the demonstration of increased activity in contralesional cortical regions during use of the affected hand using functional imaging in humans. The unaffected hemisphere is in many ways more amenable to study by TMS than the affected hemisphere, with motor thresholds that are normal and stable. Although one group reported abnormally increased MEP amplitudes early on after stroke (Delvaux et al 2003), others have reported that corticospinal excitability remains within normal limits. Some groups reported a higher than normal probability of evoking an ipsilateral (uncrossed) MEP from the unaffected hemisphere in the affected limb, but this phenomenon was only seen in severely affected patients and it is not thought that uncrossed projections play any significant role in recovery of hand function (Turton et al 1996). The situation however may be different in more proximal muscles; for example, recovery of swallowing in dysphagic patient after hemispheric stroke appears to rely mostly on expansion of control from the unaffected hemisphere (Hamdy et al 1998).

As with other TMS measures, investigations of GABAergic inhibition in the unaffected hemisphere have yielded a variety of results with the majority of reports finding normal or reduced inhibition. Interestingly, the situation may change over time. In one longitudinal study, unaffected hemisphere SICI was measured at 2 early time points; only patients who recovered well showed reduced inhibition suggesting that it may have positive role in promoting change after damage (Manganotti et al

2002). The relationship of such contralesional disinhibition to recovery, and the change in this relationship with time, is at present unclear.

1.3.4.3 Abnormalities of interhemispheric interactions

TMS can be used to measure interhemispheric interactions, most commonly between the 2 motor cortices, by using a paired pulse interaction delivered via 2 coils. At rest, the predominant effect is inhibition. After stroke, interhemispheric inhibition (IHI) from the unaffected to the affected hemisphere (UH-AH) is normal at rest but that in the other direction (AH-UH) is thought to be deficient (Murase et al 2004; Butefisch et al 2008). This is not necessarily due to direct damage to the transcallosal projections (Boroojerdi et al 1996) since it is also seen when the stroke is more caudal. A relationship has been demonstrated in such patients between reduced IHI (AH-UH) and loss of SICI within the unaffected hemisphere, suggesting that the latter may be a result of transcallosal disinhibition.

Immediately prior to the onset of voluntary movement the interhemispheric interaction switches from inhibitory to facilitatory in healthy subjects, but this switch does not occur when patients move their paretic hand (i.e. measuring IHI from UH-AH) (Murase et al 2004). This is consistent with the hypothesis that the unaffected hemisphere suppresses excitability of the affected hemisphere, thus doubly disabling its residual motor function. This has led to the idea that reducing the excitability of the unaffected motor cortex may improve recovery, and has provided the rationale for a number of therapeutic studies. Perhaps the most widely used therapy along these lines is Constraint-Induced Movement Therapy (CIMT), in which the unaffected upper limb is immobilised and intensive use of the affected limb encouraged (Taub et

al 1993). There is evidence that this approach improves paretic limb function (van der Lee et al 1999; Wolf et al 2006) and is associated with motor map expansion within the stroke hemisphere (Sawaki et al 2008).

It has not been clearly established which hemisphere is responsible for the failure to ‘switch off’ IHI; it could reside within the unaffected hemisphere or it could equally well result from an abnormality in circuits mediating IHI within the affected hemisphere. In favour of the first interpretation, a study of experimental anaesthesia of the healthy arm produced improved motor function in the paretic arm while also reducing pre-movement IHI (Floel et al 2008a). However, not all evidence supports the idea that the non-stroke hemisphere interferes with function of the damaged side. The performance of complex movements with the paretic hand is degraded by a disruptive train of TMS pulses given to the contralesional primary motor cortex (Lotze et al 2006), suggesting a positive contribution from this region. In summary, the significance of IHI targeting the lesioned hemisphere after stroke is yet to be resolved. This question may turn out to be important since individual differences in the presence of excessive interhemispheric inhibition may determine whether or not interventions that are designed to reduce contralesional excitability are successful.

TMS has been used to characterise a number of inter-regional interactions from non-primary motor regions targeting the motor cortex in healthy humans, and their roles in relation to different aspects of movement. Although a number of the cortical regions involved in these interactions show greater than normal haemodynamic activity during hand movement after stroke (Ward et al 2003a) almost nothing is known about what role, if any, such activity may play in recovered motor function. One exception

is the dorsal premotor cortex (PMd), which in healthy humans is involved in movement selection. Imaging studies have suggested that, depending on the extent of damage, either the ipsilesional or contralesional PMd may be an important contributor to recovered arm movement following stroke (Ward et al 2007). Two ‘virtual lesion’ TMS studies have shown that disruption of activity in this area increases reaction times of the paretic hand in stroke patients whereas no effect is seen in healthy subjects (Lotze et al 2006; Johansen-Berg et al 2002). The physiological interaction between the contralesional PMd and the ipsilesional motor cortex has yet to be tested after stroke.

1.3.5 Pathophysiology of stroke recovery: gaps in our understanding

As detailed above, the phenomena of reduced corticospinal excitability in the affected hemisphere and bilateral intracortical disinhibition are well recognised after stroke. However several important questions remain. The stability or otherwise of these parameters during the crucial early weeks is unclear and cannot be tested without more detailed studies during this period. While GABA_A-mediated SICI is consistently found to be abnormal, GABA_B-mediated LICI has not been tested. Previous reports differ with regard to the relationship between corticospinal / intracortical excitability and clinical recovery, and this may well be due to the time after stroke at which such studies are performed. In trying to understand mechanisms of recovery this is crucial, and can only be addressed by testing these relationships over the course of several months within the same patient group. These questions are addressed in Chapter 3.

While studies using functional imaging, and others using TMS as a ‘virtual lesion’, have hinted at a positive role for the contralesional PMd in hand movement after

stroke this is not well established. In healthy subjects, previous TMS studies suggest that this region exhibits an inhibitory interaction with the contralateral primary motor cortex at rest (Mochizuki et al 2004), and that this is modulated during movement selection (Koch et al 2006): this interaction has not been tested following stroke. Furthermore, it is unclear whether any influence of this region on movement of the paretic hand after stroke is exerted directly via an un-crossed projection from the PMd to the spinal cord or alternatively via a transcallosal interaction with the affected hemisphere. Using a novel technique in which TMS pulses are delivered within the MRI scanner during image acquisition, Bestmann et al (2008b) demonstrated in healthy subjects that the PMd interacts in a state-dependent manner with the contralateral primary motor cortex: an inhibitory interaction at rest switches to facilitation during movement of the ipsilateral (to the PMd) hand (including the author – not described in Results chapters). We have tested the nature of this interaction after stroke, using paired coil TMS to test the interhemispheric PMd-motor cortical interaction at rest and the combined TMS-MRI technique to probe changes during movement of the paretic hand: this work is described in Chapter 4.

1.4 Using non-invasive stimulation to induce plasticity in the healthy brain

1.4.1 Simple repetitive TMS

When TMS is applied in a repetitive manner it can induce changes in cortical excitability that outlast the period of stimulation. The direction of modulation and duration of effect depend on many factors, but in general it has been noted that low frequency stimulation (0.2 – 2 Hz) results in a reduction in excitability whereas high frequency (5 – 25 Hz) results in an increase. In the original low frequency study, supra-threshold stimulation at 0.9 Hz for 15 minutes reduced MEP amplitudes for 15

minutes after the period of stimulation (Chen et al 1997). A number of subsequent studies have succeeded in inducing inhibition using sub-threshold stimulus intensities, eg 90% resting threshold (Bagnato et al 2005; Chouinard et al 2003), but have noted considerable inter-individual variability in response to stimulation (Daskalakis et al 2006). Motor cortical excitability changes have also been demonstrated in response to 1 Hz stimulation of remote connected cortical regions, for example the contralateral motor cortex or premotor cortex (Heide et al 2006; Rizzo et al 2004). An increase in cortical excitability in response to high frequency stimulation was originally demonstrated by Pascual-Leone and colleagues (1994) with stimulation at 5 or 10 Hz inducing facilitation lasting 3-4 minutes. Subsequent work has demonstrated that the stimulus intensity is crucial to this facilitatory effect, with inhibition induced at lower intensities (Modugno et al 2001).

The mechanisms by which it is proposed that repetitive TMS may induce long-lasting changes in cortical excitability have in general involved synaptic plasticity. This is partly because equivalent stimulation protocols have been used in animal models to produce changes in synaptic strength but also because of a number of other striking similarities. The frequency-dependence of the outcome of repetitive TMS closely resembles the frequency-response function observed with tetanic stimulation of the Schaffer collateral projection to area CA1 of the rat hippocampus (Dudek & Bear 1992), with low frequency inhibition giving way to facilitation at higher frequencies. Both techniques may be rapidly induced, although repetitive TMS effects tend to be shorter-lived. Both depend on activity at the NMDA receptor (Fitzgerald et al 2005), can be prevented by sodium / calcium channel blocking agents (Inghilleri et al 2004a)

and modulated by activity at GABA receptors (Castro-Alamancos et al 1995; Hrabetova & Sacktor 1997; Fitzgerald et al 2005).

1.4.2 Theta Burst Stimulation in humans

The theta rhythm (5 Hz) occurs naturally in the hippocampus and has been employed artificially in animal models to induce hippocampal LTP (Larson et al 1986). It was observed in humans that short high frequency bursts of TMS pulses at low intensity, using a protocol mimicking those employed in animal experiments, could transiently increase corticospinal excitability in the motor cortex (Huang & Rothwell 2004). The basic unit of this protocol involves a burst of 3 stimuli at 50 Hz repeated at 5 Hz (ie a burst occurring every 200 ms). Subsequent studies by Huang et al (2005) revealed that this pattern given intermittently for 2 seconds in every 10 for 3 minutes induced facilitation lasting up to 15 minutes (iTBS), whereas if given continuously for 30 seconds then inhibition lasting 30 minutes was observed (cTBS). For both the continuous and intermittent forms studies in patients with cervical epidural electrodes have confirmed a cortical site of action, preferentially affecting I1 waves (continuous) or later I waves (intermittent) (Di Lazzaro et al 2005, 2008). Both forms are dependent in their action on activity at the NMDA receptor (Huang et al 2007) and can be abolished if the target muscle is contracted during stimulation (Huang et al 2008). The inhibitory and facilitatory effects of cTBS and iTBS respectively can each be reversed if stimulation is preceded by finger movements (Iezzi et al 2008). Magnetic resonance spectroscopy studies have suggested that the effects of cTBS are associated with a local increase in GABA activity, although it is not clear whether this is an epiphenomenon or central to the mechanism of action (Stagg et al 2009). It is proposed by Huang et al in the original description of TBS that the differing effects of

the 2 stimulation protocols results from 2 overlapping effects of the theta burst stimulation pattern: facilitation which is rapidly induced and inhibition which is slower in onset but stronger and dominant over longer periods of stimulation. This idea has been explored further in a recent computer model of theta burst stimulation which seeks to explain the divergent effects of different protocols in terms of the rate of calcium influx into the post-synaptic neuron, with either LTP or LTD resulting (Huang et al 2011). The short duration of stimulation required, the low intensity used (and hence relative safety) and robust effects have made TBS protocols popular in physiological studies with both healthy subjects and patients.

1.4.3 Variability in the response to stimulation

It has been a common theme in studies of repetitive TMS that there is great variability of response, both within and between individual subjects (see Pell et al 2011 for summary). Some of this variability may relate to differences in the physical characteristics of subjects and of stimulation protocol, such as skull-cortex distance (Herbsman et al 2009), coil orientation (Gerschlagier et al 2001; Tings et al 2005) and pulse waveform (Sommer et al 2002). Pulse train length (Quartarone et al 2005), stimulus intensity (Todd et al 2006) and inter-train interval (Rothkegel et al 2010) also influence the outcome of stimulation. Even with entirely consistent stimulation parameters considerable variability is observed. In attempting to explain this, there has been much interest recently in the influence of the current and recent activity state of the stimulated region on the outcome of stimulation. Such state-dependence is widely recognised in synaptic LTP/LTD induction and has more recently been investigated in human plasticity protocols, with important effects of current muscle contraction (Fujiwara & Rothwell 2004) and recent training (Ziemann et al 2004;

Stefan et al 2006). The use of a preceding period of brain stimulation in ‘priming’ the response to subsequent repetitive TMS (Iyer et al 2003) has introduced the concept of homeostatic regulation into the field of brain stimulation, with a proposed sliding threshold for synaptic modification explaining the relationship between previous and subsequent stimulation (Siebner et al 2004).

It has recently become clear that a number of other factors may also go some way to explain inter-individual variability in response to brain stimulation. These include effects of age (Muller-Dahlhaus et al 2008), gender (Kuo et al 2006), genotype for Brain Derived Neurotrophic Factor (BDNF: Cheeran et al 2008), fluctuations in hormonal levels (Inghilleri et al 2004b) and circadian rhythmicity (Cohen et al 2010). It has also recently been suggested that an individual’s capacity to modulate activity in the GABA (gamma amino butyric acid) system is important in determining their response to plasticity induction protocols (Stagg et al 2011).

1.5 Pharmacological modulation of the effects of stimulation

As detailed above, the effects of repetitive TMS on cortical excitability are felt to be dependent on changes in synaptic strength. It may therefore be expected that such synaptic plasticity would be subject to modulation by the principal neuromodulatory systems of the brain: the monoamine and cholinergic systems. Such influences have been more extensively studied with regard to the related technique of transcranial direct current stimulation (tDCS) but some information is also available for repetitive TMS protocols. The monoamine system incorporates the influences of noradrenaline (originating in the locus coeruleus), dopamine (from the ventral tegmental area) and serotonin (from the Raphe nuclei), whereas cholinergic projections originate in the

nucleus basalis of Meynert. A number of pharmacological interventions affect the monoaminergic system in a fairly non-specific manner, inhibiting synaptic re-uptake and affecting all 3 components, but specific receptor agonists / antagonists and precursors are also available.

1.5.1 The dopaminergic system

There is evidence from animals (Kanno et al 2004) and humans (Strafella et al 2003) that high frequency repetitive TMS can induce the release of dopamine in the striatum, while low frequency TMS reduces the availability of dopamine and noradrenaline (Shaul et al 2003). However the significance of these findings for potential dopaminergic modulation of repetitive TMS protocols is unclear. For tDCS, a complex influence of dopaminergic pre-medication on the outcome of stimulation has been demonstrated. Facilitatory (anodal) tDCS was inhibited by either a high or low dose of a D2 receptor agonist but not by a medium dose (Monte-Silva et al 2009). The same group further demonstrated a dose-dependent effect using the dopamine precursor levodopa, which abolished facilitatory or inhibitory tDCS at low or high doses, but at a medium dose turned the effect of normally facilitatory stimulation into inhibition (Monte-Silva et al 2010). This inverted U-shaped dose-response curve is well described for other effects of dopaminergic stimulation (Cai & Arnsten 1997; Seamans & Yang 2004) and it is proposed that a similar process may govern the effects on tDCS. There is recent evidence that dopaminergic stimulation is necessary for and may promote LTP, perhaps by boosting intracellular calcium levels (Molina-Luna et al 2009). In view of the possible role of LTP in the effects of Theta Burst Stimulation the interaction between dopaminergic stimulation and TBS is of interest.

This is investigated in healthy subjects in Chapter 5 and in patients with stroke in Chapter 6.

1.5.2 The cholinergic system

There are a limited number of previous studies investigating the effects of cholinergic modulation on plasticity induction in the human motor cortex. Differing results have been obtained with global cholinergic stimulation (using a cholinesterase inhibitor) versus specific nicotinic stimulation. Cholinesterase inhibitors are known to reduce intracortical inhibition (Korchounov et al 2005), but while they enhance the facilitatory effects of paired associative stimulation (PAS) they reduce the effects of anodal tDCS (Kuo et al 2007). Using nicotine itself, Thirugnanasambandam and colleagues (2011) found that both inhibitory and facilitatory tDCS effects were reduced or abolished, while facilitatory PAS was slightly prolonged. The authors argued that, in the case of facilitatory plasticity protocols, cholinergic stimulation (by either medication) appears to have a focusing effect, enhancing focal (PAS) but not non-focal (tDCS) forms of plasticity. There is evidence from animal studies that nicotine can produce both a pre- and post-synaptic enhancement of LTP (Fisher et al 1998; Mansvelder & McGehee 2000; Ji et al 2001; Ge & Dani 2005). In view of the possible role of an LTP-like process in the effects of TBS we therefore set out to investigate the influence of nicotine on the outcome of this form of brain stimulation: these experiments are described in Chapter 5.

1.6 The influence of brain stimulation on human motor learning

Humans are capable of remarkable and rapid learning when training in a new motor task. Our interest here is in the possibility of enhancing the response to training using

focal non-invasive brain stimulation. When attempting this it is important to understand which brain regions are being engaged in a given task. A number of motor tasks are well characterised in previous investigations.

1.6.1 Studying motor learning in human subjects

The phrase ‘motor learning’ includes a wide range of behaviours. In general 2 categories of task are studied: motor adaptation, in which subjects attempt to return a motor behaviour to normal in the face of an external perturbation, and the acquisition of a new skill: in the current work we are concerned with the latter form of learning in relation to fine movements of the hand. There is evidence from animal and human studies that the primary motor cortex plays an important role in the formation of motor memories and the acquisition of new motor tasks. LTP within the motor cortex was directly tested in rats in relation to the acquisition of a skilled task by Rioult-Pedotti et al (2000). After 5 days of training in a novel task there was a reduced capacity for LTP induction in the trained hemisphere (but not in the untrained hemisphere) but an increased capacity for LTD. This was taken both to support the involvement of motor cortical LTP in motor learning but also to suggest a sliding threshold for the induction of LTP / LTD dependent on recent synaptic activity. In humans the direction of thumb movement evoked by single TMS pulses delivered to the motor cortex can be altered by a period of repetitive practised movements in a different direction: this use-dependent plasticity implicates the motor cortex in retention of a motor memory (Classen et al 1998). Further evidence was provided by Muellbacher et al (2001) who demonstrated a muscle-specific increase in motor cortical excitability induced by practice of a ballistic thumb movement. It was subsequently demonstrated that the synchronous application of single pulse TMS to

M1 contralateral to a hand practising a thumb abduction task enhanced the ability of healthy subjects to encode an elementary and short-lasting motor memory in the primary motor cortex (Butefisch et al 2004).

Demonstrating that LTP occurs within the motor cortex, and that this region's excitability is modulated during learning, does not of course imply that this is the only region engaged in the learning process. Depending upon the task, it is very likely that changes within several cortical and sub-cortical brain regions are involved in task acquisition. In the serial reaction time task, subjects respond as quickly as possible to instructions to move one of four fingers, with implicit learning of an embedded sequence (Nissen & Bullemer 1987). In the use-dependent plasticity task described above (Classen et al 1998) subjects alter the direction of an evoked movement through practice in a different direction. Reis and colleagues have recently developed an accurate pinch grip task, in which subjects make visually-guided adjustments to pinch grip in a learned sequence (Reis et al 2009). Paired associative stimulation (PAS) is a well-characterised plasticity paradigm in which the motor evoked response to a single TMS pulse delivered to the motor cortex is conditioned by an afferent sensory stimulus timed to arrive simultaneously in the motor cortex (Stefan et al 2000). While this is not strictly a form of learning, being entirely passive, it has the advantage of providing an in vivo model of synaptic change localised to the motor cortex. The ballistic thumb movement task described by Muellbacher et al (2001) is by contrast an active process in which subjects are asked to maximise the peak acceleration of an externally-cued thumb movement. As it can be rapidly induced and is associated with excitability modulation within the motor cortex it has been used by a number of previous investigators as a model of motor learning (Ziemann et al 2004;

Agostino et al 2007, 2008; Walther et al 2008). We were interested here in the modulation of subsequent motor learning by focal non-invasive stimulation of the motor cortex, and chose this thumb movement task as our measure of motor learning.

1.6.2 The interaction of motor learning with plasticity induction paradigms

In view of the involvement of primary motor cortex in motor learning, as detailed above, it may be expected that the modulation of cortical excitability by plasticity induction paradigms may have an effect on learning outcome. When considering what effects may be expected to result from such experiments it is important to consider the stage in the learning process during which excitability is altered. In addition to changes in behaviour observed during training itself (online changes) one must consider changes observed following the completion of training (consolidation) and the long-term stabilisation of gains (retention). The process of consolidation itself incorporates the stabilisation of the motor memory (reducing susceptibility to retrograde interference) and offline gains: in contrast to stabilisation, offline gains may require the process of REM sleep (Fischer et al 2002; Walker et al 2003a; Stickgold 2005).

Non-invasive brain stimulation techniques have been employed in 2 ways in the study of motor learning: to provide details of the involvement of brain regions at various stages of the learning process, and in attempts to enhance the outcome of training. Most but not all such studies have tested modulation of the primary motor cortex, where the response to stimulation protocols is best characterised. In the first category of such studies, the application of an inhibitory repetitive TMS paradigm immediately before the onset of training had no effect on within-training changes but impaired the

retention of gains to the following day. This effect was not observed if stimulation was delivered 6 hours after training, implicating the primary motor cortex in the early consolidation process (Muellbacher et al 2002). Single TMS pulses delivered synchronously to volitional movements during training in a thumb abduction task (TMS given to the contralateral motor cortex) enhanced the encoding of a motor memory, whereas training was attenuated if the ipsilateral motor cortex was stimulated (Butefisch et al 2004). The application of an inhibitory paradigm (1 Hz repetitive TMS) to the motor cortex starting immediately after completion of training impaired consolidation in a serial reaction time task, but not if subjects subsequently slept (Robertson et al 2005). Such techniques have thus provided information regarding the role of the primary motor cortex in training itself and during subsequent consolidation.

Physiological studies in humans and cortical mapping experiments in animals have demonstrated that motor learning is associated with an increase in excitability in the primary motor cortex contralateral to the training hand (Nudo et al 1996; Muellbacher et al 2001). On this basis, it has been suggested that artificially increasing motor cortical excitability may enhance the outcome of learning. Some (Schambra et al 2003; Plewnia et al 2003) but not all (Wassermann et al 1998) investigators have found that cortical excitability can be increased alternatively by applying an inhibitory paradigm to the contralateral motor cortex, providing a further means of increasing excitability in the ‘target’ motor cortex by transcallosal disinhibition. Some studies targeting the motor cortex have been encouraging: training in a motor sequence task has been successfully enhanced using 10 Hz repetitive TMS (Kim et al 2004) and by anodal tDCS (Vines et al 2006), which also improved implicit sequence learning

(Nitsche et al 2003). Others have not demonstrated a benefit, with no effect seen on the learning of rapid finger movements from sub-threshold stimulation at 5Hz (Agostino et al 2007) and it may be that the choice of task is important. One study has demonstrated improved acquisition of sequential finger movements with inhibitory stimulation of the ipsilateral motor cortex (Kobayashi et al 2004).

1.6.3 Considerations from a homeostatic perspective

It is not necessarily the case, however, that increasing motor cortical excitability should necessarily enhance the response to training. The observation, discussed above, that motor learning in rats reduces the capacity for subsequent LTP induction gave rise to a number of experiments in humans examining such a ‘homeostatic’ response to previous plasticity induction. It is now well established that such considerations do in fact crucially affect the outcome of plasticity induction paradigms, including training. Thus when cathodal tDCS was used to ‘prime’ the motor cortex with an inhibitory stimulus the effect of subsequent 1 Hz TMS (usually inhibitory) was reversed to facilitation (Siebner et al 2004). Using a similar priming approach, the response to 1 Hz TMS can be enhanced by preceding high frequency TMS (Iyer et al 2003), while the direction of change in response to 20 Hz stimulation can be defined by the polarity of preceding tDCS (Lang et al 2004). Using PAS as a marker of LTP-like or LTD-like plasticity, it has been possible to test the interaction between synaptic plasticity and motor learning. 2 studies have suggested that there is a temporary reduction in the capacity for LTP soon after learning, with increased capacity for LTD, in line with the animal data (Stefan et al 2006; Rosenkranz et al 2007). The converse has recently been examined, with PAS preceding motor learning, revealing that such an interaction depends crucially upon the time interval. The

predicted homeostatic interaction was observed when LTD-like PAS immediately preceded training (learning was enhanced), and when either LTD-like or LTP-like PAS preceded training by 90 minutes (learning enhanced or attenuated respectively), but the reverse was observed when LTP-like PAS immediately preceded training: learning was again enhanced (Jung & Ziemann 2009). The authors distinguish on this basis between homeostatic and non-homeostatic interactions, arguing that in some cases a non-homeostatic interaction can predominate. Other authors have observed interactions between tDCS and subsequent learning of a serial reaction time task that do not obey such homeostatic predictions, suggesting that the outcome of behavioural learning tasks may be difficult to predict according to such rules (Kuo et al 2008b).

We were interested here in the interaction between facilitatory Theta Burst Stimulation applied to the primary motor cortex and the subsequent acquisition of a ballistic thumb movement task. In particular, we asked whether such an approach could enhance learning (or whether homeostatic rules would perhaps inhibit it) and whether the effects on cortical excitability would match those on learning. We included cholinergic modulation by nicotine: see 1.6.2 above for discussion. These questions are explored in experiments described in Chapter 5.

1.7 Applying brain stimulation to enhance motor training after stroke

1.7.1 What should we expect when modulating excitability before training?

As discussed above, non-invasive brain stimulation can induce focal modulation of cortical excitability and can influence the outcome of motor training. This has opened the door to the attractive possibility of using brain stimulation as a therapy to improve motor recovery from hemiparesis after stroke. However the interaction of artificial

plasticity protocols with learning in healthy subjects is not straightforward, and in the context of impaired corticospinal excitability it is not entirely clear what one would expect to see. It may be that restoring excitability towards the normal range would encourage changes in synaptic strength to occur, resulting in a synergistic interaction allowing performance gains to be stabilised. On the other hand, as detailed above, stimulation protocols inducing LTP-like changes may raise the threshold for further changes, resulting in a homeostatic interaction and impaired learning. Here we review the outcome of attempts to use non-invasive brain stimulation to enhance motor performance and learning after stroke, and discuss the techniques used to address this issue in the current work.

While early studies along these lines have demonstrated behavioural improvements resulting from brain stimulation (Hummel & Cohen 2005; Talelli et al 2007) the transient nature of the induced effects limits this approach. More recently investigators have sought to use stimulation to ‘prime’ patients for rehabilitation training in the hope of enhancing training outcome, thereby inducing lasting improvements. These investigations can be divided into those aiming to increase excitability in the motor cortex of the affected hemisphere and those aiming to reduce it in the unaffected hemisphere: the rationale of the latter approach is to reduce transcallosal inhibition of the affected side by the unaffected side (Murase et al 2004; Ward & Cohen 2004). The therapeutic technique of CIMT, as described in section 1.3.4.3 above, may be seen as attempting to achieve both of these aims.

1.7.2 Enhancing excitability of the affected motor cortex

As detailed above, corticospinal excitability in the affected hemisphere is frequently reduced after either cortical or subcortical stroke. The approach of enhancing excitability by non-invasive stimulation is limited in the case of significant cortical strokes by the extent of surviving cortical tissue, the presence in the acute phase of oedema and metabolic disturbance, and the small risk of inducing seizures. Ameli and colleagues (2009) found that 10 Hz stimulation applied to the motor cortex in a group of 29 chronic stroke patients only induced improved movement kinematics in those with subcortical stroke. Therefore studies in this field tend to include either patients with exclusively subcortical strokes or those in which the motor cortex is spared. Some studies along these lines have produced promising results. Khedr et al (2005) applied 3 Hz suprathreshold stimulation to the motor cortex in the acute period over a 10 day period as an adjunct to conventional physiotherapy and demonstrated significant gains across a range of clinical and neurophysiological scales, although not in patients with large middle cerebral artery infarcts. A follow-up study demonstrated sustained (though modest) benefits of stimulation in the acute period when tested again at 1 year (Khedr et al 2010). Kim and colleagues (2006) used a high frequency stimulation protocol in patients with chronic stroke prior to training in a complex sequential finger task and found that the effect on excitability was positively associated with the outcome of training, suggesting that this approach is feasible in the chronic phase.

1.7.3 Reducing excitability in the unaffected motor cortex

A number of studies have now demonstrated beneficial effects from stimulation of the motor cortex in the intact hemisphere. This is in many ways a more attractive therapeutic target, with anatomically intact structures and very little in the way of

seizure risk. Initial studies looked at the effect of 1 Hz stimulation on movement kinematics in patients with chronic stroke, and demonstrated transient improvements (Mansur et al 2005; Takeuchi et al 2005). Fregni and colleagues (2006) demonstrated that such improvements were greater when patients were treated on 5 consecutive days and that kinematic improvements were sustained at 2 weeks. A similar protocol applied over a single session in combination with functional MRI resulted again in improvement finger and grasp movements in the paretic hand which were associated with normalisation of the motor activation pattern (Nowak et al 2008). Grefkes and colleagues (2010) took this approach further: they used Dynamic Causal Modeling to demonstrate that improved fist closure movements following 1 Hz TMS to the unaffected motor cortex were associated with a reduction in the negative influence of the stimulated region on activity in the motor cortex of the affected hemisphere. Takeuchi and colleagues (2008) used a similar TMS protocol to prime patients with chronic stroke for training in a pinching task with the paretic hand, resulting in an excitability increase in the affected hemisphere and greater improvement in pinch force with training, stable at 1 week. Khedr and colleagues (2009) applied a similar protocol in the acute period after stroke, with stimulation on 5 consecutive days: they found that clinical measures of motor function were improved over sham, and that 1 Hz applied to the unaffected hemisphere was superior to 3 Hz applied to the affected motor cortex. There is therefore accumulating evidence in favour of this approach inducing behavioural improvements after stroke, which may to some extent be stable.

1.7.4 Dopaminergic modulation after stroke

As discussed above, dopaminergic stimulation interacts in a non-linear dose-dependent manner with the effects of both transcranial direct current stimulation and

Paired Associative Stimulation in healthy subjects, and in animal models can promote synaptic LTP (Molina-Luna et al 2009). The effects of combining dopaminergic stimulation with Theta Burst Stimulation, a stimulation protocol which also exhibits LTP-like properties, are hitherto unknown but are explored in the following chapters. Beneficial effects of dopaminergic stimulation have been demonstrated in both motor and non-motor forms of learning in humans. Use-dependent plasticity is enhanced in the presence of the dopamine agonist cabergoline but attenuated by the antagonist haloperidol (Meintzschel & Ziemann 2006). A similar effect has been observed in this plasticity paradigm in response to levodopa by Floel et al (2005a) in a group of patients with chronic stroke, while improved procedural learning of a serial reaction time task is also reported (Rosser et al 2008). It is also suggested that exogenous levodopa may boost depleted striatal dopamine levels in otherwise healthy elderly subjects, with consequent improvement in motor function (Floel et al 2008b). With regard to the effect on motor training it has been proposed that dopaminergic stimulation selectively boosts activity at NMDA glutamatergic receptors relative to other excitatory receptors, enhancing the signal-to-noise ratio and thereby enhancing synaptic changes in response to training (Cepeda et al 1992).

A number of studies have investigated the clinical use of dopaminergic agents during stroke recovery, with variable results. While Scheidtmann and colleagues (2001) reported in a double-blind clinical study that levodopa was associated with significant gains in clinical motor scores relative to placebo, other investigators have struggled to reproduce this finding: a meta-analysis of clinical trials has concluded that there is insufficient evidence to support the clinical use of levodopa after stroke at present (Berends et al 2009).

1.7.5 Potential advantages of Theta Burst Stimulation

From the discussion above it will be evident that extensive investigations into brain stimulation as a potential therapeutic adjunct after stroke have produced promising but mixed results. We were interested here to investigate the use of TBS to prime patients with stroke for motor learning for a number of reasons. The low stimulus intensity used in this form of stimulation (80% of active motor threshold) avoids the potential seizure concerns associated with higher intensities. It also results in more focal stimulation, with less possibility of spread to adjacent regions. This fact should also result in more effective sham stimulation in a double blind study. Finally, the facilitatory form of TBS can be delivered in around 3 minutes: this short duration is advantageous in view of the critical time relationship between brain stimulation and training discussed above. Talelli and colleagues (2007) demonstrated that TBS applied to the affected hemisphere in patients with chronic stroke is safe and feasible, producing transient kinematic improvements, but the effect on motor learning in patients is unknown. A double-blind placebo- and sham-controlled trial testing the effects of TBS with or without levodopa on subsequent motor learning in a group of stroke patients is described in Chapter 6.

Chapter 2

Methods

2.1 Participants

Healthy subjects for normative studies were recruited from a database of healthy volunteers at the Institute of Neurology (for work carried out in London) and from an equivalent database within the National Institutes of Health (for work carried out in the US). All healthy subjects were right-handed non-smokers on no regular medication. Patients with chronic stroke were recruited from patient databases held within the Institute of Neurology (Chapters 4 & 6) and the National Institute of Neurological Disorders and Stroke (Chapter 6). Patients with acute stroke (Chapter 3) were recruited from the Acute Stroke and Brain Injury Unit of the National Hospital for Neurology and Neurosurgery, London. Please see individual chapters for patient inclusion and exclusion criteria.

2.2 Institutional and ethical approval

All studies performed within the Institute of Neurology (London) were approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, UCL Hospitals NHS Trust, London. The study performed at the National Institute of Neurological Disorders and Stroke (NINDS, Bethesda, Maryland, United States of America – see Chapter 6) was approved by the NINDS Institutional Review Board, while the use of Transcranial Magnetic Stimulation for Theta Burst Stimulation in human subjects received specific approval from the Food and Drugs Administration (FDA). For all studies, participants gave written informed consent. All studies were performed in accordance with the Declaration of Helsinki.

2.3 Assessment of motor function in patients with stroke

The following scales of motor function were used in our patient groups: Nine Hole Peg Test (NHPT), Action Research Arm Test (ARAT), Motricity Index and the Jebsen Taylor Test (JTT). Further scales of global clinical function were also employed – please see individual chapters. The NHPT involves measuring the time taken to place nine pegs consecutively into individual holes, using one hand. If the task was not completed within a minute then the number of pegs placed within that time was scored. Each hand was examined 3 times at each evaluation, and the mean score recorded as pegs per second for that hand. In the ARAT, patients are asked to manipulate objects of varying sizes with the affected arm, assessing 4 aspects of arm function: grasp, grip, pinch movements and gross arm movements. The ARAT and NHPT were each assessed on both the affected and unaffected sides, and were subsequently corrected within each patient by expressing the score for the affected side as a percentage of that for the unaffected side, such that no impairment at all would result in a score of 100%. The Motricity Index reflects a clinical assessment of power in 3 muscle groups of the upper limb. For the JTT, patients were timed performing six tasks as fast as possible with the affected upper limb: turning cards, picking up pennies, using a spoon, stacking checkers, moving light cans and moving heavy cans. Each stage was performed 3 times for every assessment and the average total time taken for all 6 components determined (expressed in seconds).

2.4 Transcranial Magnetic Stimulation (TMS)

2.4.1 TMS Coils and magnetic stimulators

For single- and paired-pulse TMS experiments, either one or two monophasic Magstim 200 stimulators were employed (The Magstim Company, Dyfed, UK)

connected by a Y-cable to a single figure-of-eight shaped coil with a 70mm internal wing diameter. In Chapter 4 a smaller coil was used to stimulate the dorsal premotor cortex. For delivering Theta Burst Stimulation (see 2.4.5 below) a Magstim Rapid biphasic stimulator was instead used. For all TMS experiments subjects were seated comfortably in an armchair and were asked to remain still (where appropriate) and silent, while the investigator stood behind holding the stimulator coil.

2.4.2 Motor threshold measurement

For experiments targeting the primary motor cortex the coil was held with the handle pointing postero-laterally at an angle of 45 degrees to the midline, such that the current induced in the brain was in an anterior direction. The position was identified at which stimulation produced optimal Motor Evoked Potentials (MEPs) in the contralateral target muscle (usually the First Dorsal Interosseus) and this position was used for the remainder of the experiment. The resting motor threshold (rMT) was defined as the lowest stimulation intensity required to evoke an MEP in the relaxed target muscle of >50 mV in 5 out of 10 trials. The active motor threshold (aMT) was defined as the lowest stimulation intensity required to evoke an MEP in the slightly activated target muscle (10-20% of Maximum Voluntary Contraction) of > 200 μ V in 5 out of 10 trials.

2.4.3 Recording of evoked responses

Surface electromyography (EMG) was obtained using a belly-to-tendon montage from the target hand muscle. The raw signal was amplified and filtered with a band-pass filter (Digitimer Ltd; typically 30 Hz to 1 kHz - see individual chapters). Signals

were digitized at 2 kHz (CED Power1401, Cambridge Electronic Design, Cambridge, UK) and stored on a laboratory computer for offline analysis.

2.4.4 Paired pulse TMS parameters

The following intracortical excitability parameters were obtained using paired pulse stimulation through a single coil: Short Interval Intracortical Inhibition (SICI), Intracortical Facilitation (ICF) and Long Interval Intracortical Inhibition (LICI). For SICI and ICF, a sub-threshold conditioning stimulus intensity at 80% active motor threshold preceded the suprathreshold test stimulus by a variable interstimulus interval: 2 ms and 3 ms for SICI; 10 ms and 15 ms for ICF. Trials were performed at an average interval of 5 seconds, with conditions randomly intermixed. For LICI, 2 suprathreshold stimuli were delivered separated by 100 ms. See individual chapters for details of stimulus intensities. Interhemispheric interactions were studied using 2 coils. The test stimulus was delivered to the primary motor cortex contralateral to the target muscle. When testing the interhemispheric interaction between the dorsal premotor cortex (PMd) and motor cortex (Chapter 4) an interstimulus interval of 8 ms was used.

2.4.5 Theta Burst Stimulation (TBS)

For experiments investigating the effects of TBS applied to the human motor cortex, the intermittent TBS (iTBS) paradigm described by Huang et al (2005) was employed. Bursts consisting of 3 pulses at 50 Hz, at an intensity of 80% aMT, were repeated every 200 ms (ie 5 Hz) for 2 seconds. This 2 second train was repeated once every 10 seconds for 20 repetitions, a total of 192 seconds. This stimulation protocol produces an increase in corticospinal excitability lasting up to 15 minutes.

2.5 Functional Magnetic Resonance Imaging (MRI)

2.5.1 MRI data acquisition

MRI was conducted with a 1.5T Magnetom Sonata system (Siemens Medical Solutions, Erlangen, Germany), operating with the standard CP receive head and body transmit coil. Whole-head T1-weighted structural anatomical images were acquired after the fMRI experiment using a 3D MDEFT sequence with an isotropic resolution of 1 mm³ (Deichmann et al 2004). During fMRI, functional T2*-weighted MRI transverse echo-planar images (EPI) with blood oxygenation level dependent (BOLD) contrast were obtained using a multi-slice gradient echo EPI sequence with the following parameters: 848 volume acquisitions, 20 slices / volume, 64 x 96 matrix, 3 x 3 mm in-plane resolution with 50% oversampling in phase-encoding direction, 2.5 mm slice thickness plus 50% spatial gap between spatially adjacent slices, repetition time (TR)=1800 ms; TE=42 ms; $\alpha=90^\circ$; echo-spacing 500 μ s; 2298 Hz/pixel bandwidth; trapezoidal readout gradients with a ramp of 130 μ s and a flat top of 240 μ s; field of view: 192 x 192 mm; max slew rate 214.9 mT/m/ms. During scanning, any potential physiological or technical artifacts were constantly monitored online (Weiskopf et al 2007). After each experimental session, whole brain coverage EPI volumes were acquired in the same orientation as for the actual experiment, to facilitate spatial normalisation of the spatially restricted functional image series.

2.5.2 Technical aspects of concurrent TMS-fMRI

TMS was implemented inside the scanner using a MagStim Rapid system (The Magstim Company, Dyfed, UK) with a custom-built MR-compatible, non-ferrous figure-of-eight stimulation coil (two windings of ten turns each; inner wing diameter

53 mm, distance between outer coil surface and windings of 2-3 mm [variation due to manufacturing tolerance]; coil inductance, including cable, of 20 μ H; maximal current at 100% stimulator output of \sim 5kA). The stimulation unit was housed inside the scanner room in a shielded cabinet, from which the stimulation coil cable was fed through a custom filter box (The Magstim Company). Residual RF transmission along the coil cable was further suppressed using ferrite sleeves (Bestmann et al 2008a; Blankenburg et al 2008; Ruff et al 2006). The TMS coil was connected to the stimulator in parallel to a high voltage relay-diode combination (Magstim ES9486, The Magstim Company), eliminating residual leakage current flow through the TMS coil (Bestmann et al 2008b; Weiskopf et al 2009). The relay and TMS stimulator were controlled with a unit developed in-house based on a BASIC Stamp 2 micro-controller (Parallax Inc., Rocklin, California, USA). Visual stimulation, grip-force data acquisition, TMS triggering and intensity regulation, and relay settings were controlled using the toolbox Cogent 2000 (<http://www.vislab.ucl.ac.uk/cogent.php>) running under Matlab (The Mathworks, Natick, Massachusetts, USA). Foam-padded cushions were used to restrict head-movements. Participants wore earplugs (SNR=36dB) and headphones to reduce acoustic noise from the scanner and the TMS discharge sound. Inside the scanner, accurate placement of the TMS coil was ensured using an MR-compatible custom-built coil holder.

2.6 Administering medication

Experiments with healthy subjects (Chapter 5) employed Nicotine, Levodopa and Dextro-amphetamine. For Nicotine, subjects were given 2 x 2 mg Nicotine mint lozenges (active medication) or 2 inert mint lozenges (placebo), in both cases combined with a Fisherman's Friend strong tasting mint in order disguise any taste

associated with nicotine. For Levodopa and Dextro-amphetamine subjects were given tablets prepared by the National Hospital for Neurology and Neurosurgery pharmacy containing either dextro-amphetamine 10 mg, ascorbic acid 100 mg or levodopa / carbidopa in the form of Madopar 100/25 mg. The experiment performed in patients with stroke (Chapter 6) also employed Levodopa. In this case, patients took either Carbidopa-Levodopa (100mg-25mg) or a placebo preparation (starch powder and magnesium stearate in a pink opaque capsule). Both preparations were dispensed and packaged by the pharmacy of the Clinical Centre in the National Institutes of Health.

2.7 Thumb movement task

In order to test acquisition of a new motor task in healthy subjects (Chapter 5) and patients with stroke (Chapter 6) we used a modified version of a well-characterised task in which behavioural improvement is associated with physiological changes in the primary motor cortex (Muellbacher et al 2001). The subject's hand was positioned supine on a board with the wrist, metacarpophalangeal and distal interphalangeal joints fixed with Velcro straps. The thumb was left unsecured and could abduct and oppose freely. A monoaxial accelerometer (see Chapters 5 & 6 for models) was attached on the lateral aspect of the left thumb proximal phalanx with the maximal vector being thumb abduction. The accelerometer signal was sampled (CED 1401; Cambridge Electronic Design, Cambridge, UK) and not filtered. Subjects were asked to perform ballistic thumb abduction movements in time with a 0.5Hz audio metronome, with the explicit aim of maximising the initial peak acceleration in the chosen direction. The computer monitor provided online visual feedback (see Chapters 5 & 6 for details).

2.8 Data analysis

2.8.1 TMS parameters

EMG recordings were stored within Signal software (Cambridge Electronic Design, Cambridge, UK). Individual trials were examined offline and any with EMG activity prior to the TMS stimulus artifact were discarded. Peak-to-peak MEP amplitudes were measured in the remaining trials using a customised script and exported to Excel (Microsoft Corporation, Seattle, USA) for further analysis. Within each experimental condition outliers (defined as > 2 standard deviations from the mean) were removed and the mean determined. For paired pulse parameters the interaction was determined as (conditioned amplitude / unconditioned amplitude) – see individual chapters for details of interstimulus intervals and stimulus intensities.

2.8.2 Functional MRI data

Echoplanar (EPI) images were reconstructed offline (Josephs et al 2000) and the first five volumes discarded to allow for T_1 equilibration effects. All EPI slices coinciding with TMS pulses, and any additional spurious image slices (0.12% of all slices) were inspected using the ArtRepair toolbox implemented in SPM5 (<http://cibsr.stanford.edu/tools/ArtRepair/ArtRepair.h>). These rare spurious slices were replaced by interpolation between the previous and subsequent acquisition of the same slice (Bestmann et al 2008b; Ruff et al 2006). Functional imaging data were analysed using Statistical Parametric Mapping (SPM5, <http://www.fil.ion.ucl.ac.uk/spm>) implemented in Matlab 7. Realignment to the first volume corrected for any inter-scan head movements. Interactions of head motion with geometric distortions were accounted for using the ‘unwarp’ toolbox as implemented in SPM5 (Andersson et al 2001). Additional preprocessing included de-

trending of time-series in each voxel with a linear model of the global signal (Macey et al 2004) and an AR(1)-model to account for serial autocorrelations in the data. The resulting images were spatially normalised to a standard EPI template based on the Montreal Neurological Institute (MNI) reference brain in Talairach space (MNI305 brain), using 4th-degree b-spline interpolation, and re-sampled to a 3 x 3 x 3 mm³ voxel size. Spatial normalisation parameters were estimated from the whole-brain EPI images, and the respective normalisation transformation was then applied to the EPI images of the main experimental session. The resulting images were smoothed with an isotropic 9 mm FWHM Gaussian kernel, to allow for valid statistical inference according to Gaussian random field theory, in accord with the standard SPM approach. Any potential remaining artefacts related to head motion or other non-physiological signals were then removed using automatic ICA-based ‘denoising’ (Tohka et al 2008). Statistical analysis of the fMRI data involved 2 stages: first, a single subject fixed-effects model was computed for each participant by multiple regression of the voxel-wise time-series onto a composite model containing the covariates of interest; second, a group level random effects analysis comprised parameter estimates for each contrast tested across all subjects. Please see Chapter 4 for details of contrasts tested, statistical thresholds and further secondary analyses of the fMRI data.

2.8.3 Analysis of thumb movement task

Accelerometer traces were stored within Signal software (Cambridge Electronic Design, Cambridge, UK). Trials were individually reviewed to remove any containing premature movement. A custom script was used to identify the peak of the first positive deflection in the plane of interest: this was adjusted manually for every trial

to ensure precision and the peak accelerations for each trial recorded and exported for further analysis. Please see Chapters 5 & 6 for details of further analyses.

2.8.4 Statistics

Statistical analysis of TMS and behavioural data was performed using the SPSS package (IBM Corporation). Patient physiological data sets were tested for normal distribution using the Kolmogorov-Smirnoff test (Chapter 3). Effects of time, experimental condition or intervention were tested using repeated measures analysis of variance (ANOVA). When significant interactions between factors under test were observed experimental conditions were compared using the student's t-test (paired or un-paired as appropriate). Correlations between independent variables were tested using linear regression analysis producing a Pearson correlation coefficient. When testing the significance or otherwise of a correlation coefficient (r), the statistic

$$t = \frac{r}{\sqrt{\frac{1-r^2}{N-2}}}$$

was assumed to be distributed as t with $N-2$ degrees of freedom. When testing the directional variability of evoked thumb movements in Chapter 5 the concentration parameter (κ , a measure of directionality of the distribution) was derived using the circular statistics software Oriana (Oriana for Windows, Kovach Computing Services, Anglesey, Wales). Statistical analysis of functional MRI data is described separately – see above. Please see individual chapters for details of any additional statistical tests.

Chapter 3

Stages of motor output reorganisation after hemispheric stroke: longitudinal TMS study

Work described in this chapter was published in Cerebral Cortex:

Swayne OB, Rothwell JC, Ward NS, Greenwood RJ (2008) Stages of motor output reorganization after hemispheric stroke suggested by longitudinal studies of cortical physiology. Cereb Cortex 18(8):1909-22.

3.1 Introduction

Ischaemic stroke frequently leads to impairment of upper limb motor function, after which a variable degree of motor recovery is seen (Twitchell 1957). Functional imaging in humans (Ward et al 2003a, 2004) and physiological observations in animal models (Jones & Schallert 1994; Nudo & Milliken 1996) suggest that recovery of function is associated with extensive reorganisation of the motor system at the cortical level, presumably to maximise control of remaining motor output. Transcranial magnetic stimulation (TMS) has also been used in human stroke patients to probe corticospinal and intracortical physiology. Reduced corticospinal excitability from the affected hemisphere reflects damage to the corticospinal connection (Traversa et al 1998; Byrnes et al 2001) whilst increased intracortical excitability in both hemispheres (Liepert et al 2000a, 2000b; Manganotti et al 2002) reflects changes in intrinsic circuits of the cortex.

There are, however, important gaps in our knowledge. First, physiological data acquired during the first weeks after stroke have not provided consistent results: motor thresholds in the affected hemisphere were raised in some (Liepert et al 2000a; Manganotti et al 2002) but not all (Delvaux et al 2003) studies. Likewise, corticospinal hyperexcitability in the unaffected hemisphere was observed in some studies (Cicinelli et al 1997; Traversa et al 1998; Delvaux et al 2003) but not in others (Manganotti et al 2002). For many patients the early days and weeks after stroke are likely to be a period of great clinical and physiological change. Although several studies have performed TMS assessments during this early period, many have made only a single assessment. The greatest number within the first month is 3 measures of corticospinal excitability (D'Ohlaberrigue et al

1997; Delvaux et al 2003) and 2 measures of intracortical excitability (Manganotti et al 2002). Given the variability in many physiological parameters found by previous studies, it seems likely that more frequent early measurements would provide a more accurate assessment of early neurophysiological changes after stroke.

Secondly, little is known about the clinical significance of these abnormalities. Reduced corticospinal excitability of the affected hemisphere in the first 5 days after stroke predicts poorer motor outcome later on (Trompetto et al 2000) and is known to be associated with poor function when studied in the chronic stage (Thickbroom et al 2002). Likewise, intracortical disinhibition of the affected and unaffected hemispheres (at one month) is seen in patients with greater motor impairment (Manganotti et al 2002). Thus, although some neurophysiological parameters appear to be related to motor impairment in these cross-sectional studies, it is not clear whether a longitudinal relationship exists. Furthermore, these measures assess different aspects of neurophysiological function each of which might be more or less important for recovery at different times after stroke. It is therefore important to know whether the relationship between motor impairment and these parameters changes during the days, weeks, and months after stroke.

We present experiments in which we acquired detailed longitudinal neurophysiological and clinical data over the first few weeks and months after first ever ischaemic stroke. Our patient group had a relatively wide range of functional impairment, allowing the possibility to examine correlations with clinical scores. Single pulse TMS measures (resting and active motor thresholds, and motor evoked potential recruitment curves)

provide information about corticospinal excitability, specifically of the remaining original projection from the primary motor cortex to the spinal cord. The three paired pulse measures used (short and long interval intracortical inhibition, and intracortical facilitation) are well-described parameters which provide information about intracortical excitability. These intracortical interactions are thought to play a role in regulating the output of the motor cortex. We reasoned that if dynamic changes occur in how the motor output is organised during the course of recovery, these would likely be reflected in corresponding changes in the relationships between physiological parameters and behavioural measures.

We included multiple time points during the early post-stroke period to provide information about the variability of neurophysiological parameters at this stage, the degree to which early abnormalities might resolve, and the time course over which this might occur. Our aim was to provide a picture of the changes in cortical physiology occurring after stroke in this group of 10 patients, and to relate these changes to upper limb function. On the basis of data from this group we propose a model, for further testing, of how cortical reorganisation may facilitate functional recovery.

3.2 Study design

We aimed to begin the assessments as soon as possible after stroke in order to gain insight into the pathophysiology of the acute period. TMS studies as described below, along with clinical assessments, were performed as close to daily as possible for the first week after recruitment. Thereafter assessments occurred weekly until one month after

the stroke, and were then repeated at 3 and 6 months. The exact timings were dictated by the practicalities associated with the patient's care: in some cases fewer sessions were possible during the first week of participation. Figure 3.1 shows the ideal experimental timeline alongside that obtained.

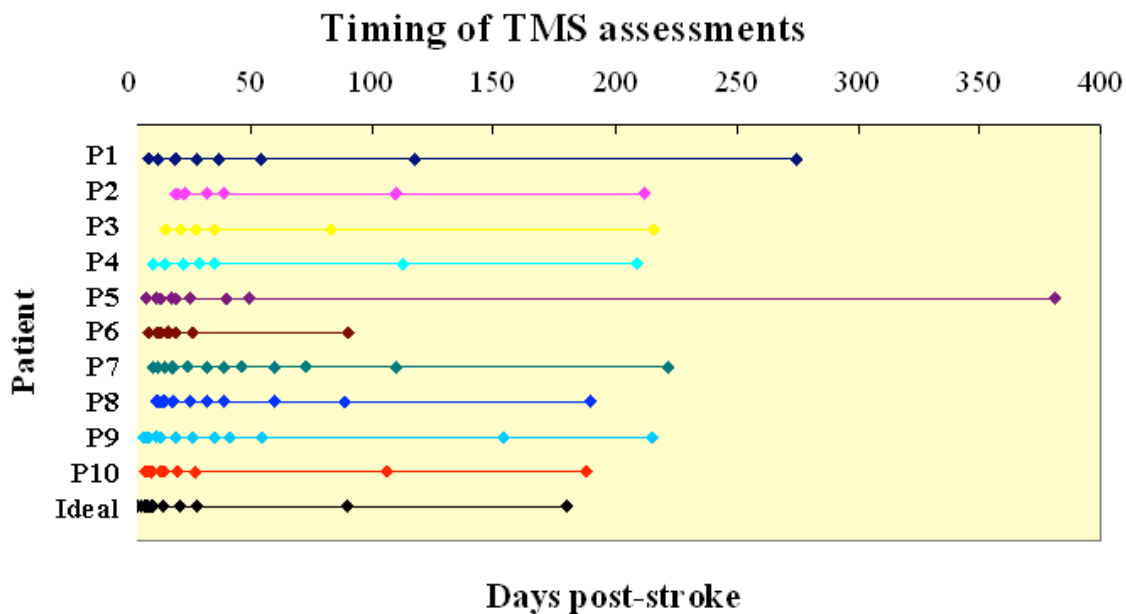


Figure 3.1. Time points at which TMS assessments were performed for the 10 patients are shown alongside the ideal schedule. Patient 5's 6 month follow-up was delayed due to unavailability. One patient was unavailable for assessment at the 3 month time point and another was unavailable at 6 months; otherwise complete data sets were obtained in all 10 patients.

3.2.1 Patient recruitment

10 patients were recruited consecutively from the Acute Stroke and Brain Injury Unit of the National Hospital for Neurology and Neurosurgery, London. All patients had suffered from first ever ischaemic stroke causing upper limb weakness (4 or less on the Medical Research Council (MRC) scale in at least one upper limb muscle group) lasting

more than 48 hours. Exclusion criteria consisted of 1) history of major psychiatric or previous neurological disease, including seizures or previous stroke; 2) cognitive impairment or dysphasia sufficient to affect informed consent; and 3) major co-morbidity. All patients received multi-disciplinary post-stroke care appropriate to their clinical needs. This included a 45 minute physiotherapy session every weekday wherever possible of their in-patient stay. Routine clinical MR imaging comprising T1, T2, FLAIR and Diffusion Weighted sequences was performed in all patients within a week of the stroke. Only patients with ischaemic infarction were included, as the vascular pathology of intracerebral haemorrhage may result in different pathophysiological changes during the acute period studied. Patients were not selected on the basis of lesion site. The age-matched control group of 10 subjects was recruited from a database of volunteers held at the Institute of Neurology, London. They were right-handed according to the Edinburgh Inventory of handedness (Oldfield 1971), with a mean handedness score of +19.1, and reported no history of neurological or psychiatric illness.

3.2.2 Behavioural assessment

On each occasion, patients were evaluated using a battery of standardised outcome measures designed to assess both upper limb function (Action Research Arm Test, ARAT; and Nine-Hole Peg Test, NHPT) and more global aspects of recovery (Motricity Index; National Institutes of Health Stroke Scale, NIHSS; timed 10 metre walk; Barthel Index; and Modified Rankin Score). See Chapter 2 for details of NHPT and ARAT. The ARAT and NHPT were each assessed on both the affected and unaffected sides, and were subsequently corrected within each patient by expressing the score for the affected side as

a percentage of that for the unaffected side, such that no impairment at all would result in a score of 100%. While the ARAT tests a range of aspects of arm and hand function, including proximal movements, the NHPT depends largely on the fine control of finger movements.

3.2.3 TMS studies

Single and paired pulse TMS measures were obtained using 2 stimulators connected via a Y-cable to a single figure-of-eight-shaped coil. TMS was delivered to the primary motor cortex, as located by identifying the motor hotspot for that hemisphere. In the unaffected and affected hemispheres (UH and AH respectively), the following parameters were assessed: resting motor threshold (rMT); active motor threshold (aMT); MEP recruitment curves, Short Interval Intracortical Inhibition (SICI – interstimulus intervals (ISIs) 2 ms & 3 ms); Intracortical Facilitation (ICF – ISIs 10 ms & 15 ms); and Long Interval Intracortical Inhibition (LICI – ISI 100 ms). For recruitment curves, MEPs were recorded in turn at 90%, 110%, 130% and 150% of the rMT for that hemisphere. SICI and ICF were measured with a conditioning stimulus (CS) fixed at 80% of aMT and a test stimulus intensity adjusted to elicit an un-conditioned MEP of 1 mV (SI-1mV). For LICI, SI-1MV was used for both CS and TS. For the SICI/ICF and LICI experiments, if it was not possible to evoke an MEP of 1 mV amplitude due to impaired MEP recruitment, then the lowest stimulus intensity resulting in an MEP of stable size was used.

3.2.4 Data analysis

When examining correlations between motor thresholds and clinical scores it was desirable to minimise the effect of the considerable between-subject variability commonly observed in the healthy population. We therefore normalised thresholds in the affected hemisphere to those in the unaffected hemisphere at each data point, since natural variability is commonly symmetrical. The formula used was (affected hemisphere / unaffected hemisphere – 1), such that raised thresholds in the affected hemisphere would result in a positive value, while the converse situation would result in a negative value. For the recruitment curve data, the mean MEP amplitude was calculated at each stimulus intensity and a curve plotted of MEP amplitude versus stimulus intensity. If different stimulus intensities were used (due to a high motor threshold) then their relation to rMT was calculated and the recruitment curve plotted in the usual way. The recruitment curve gradient was determined for each hemisphere by calculating the gradient of the line of best fit. As only 4 stimulus intensities were tested, a linear fit was applied using the least squares method, in which a line $y = mx + b$ is determined using the formula:

$$m = \frac{\sum (x - \bar{x})(y - \bar{y})}{\sum (x - \bar{x})^2}$$

When calculating SICI and ICF, the (conditioned / unconditioned MEP amplitude %) was determined for each interstimulus interval: SICI was calculated as the mean of these values for ISIs 2ms and 3ms, and ICF as the mean for 10ms and 15ms.

For LICI, the mean MEP amplitude resulting from the second (test) stimulus was expressed as a percentage of that resulting from the first (conditioning) stimulus.

Considerable within-subject variability was observed in the physiological parameters during the first month after stroke (see section 3.4 below). It was therefore desirable to define a measure representative of this period for each patient, in order that clinical correlations from this period could be examined for each parameter. We defined the ‘acute period’ measure, in each patient, as the mean of all values obtained for that parameter within the first 3 weeks after stroke. This time period was chosen so that the early weeks may be effectively represented while maintaining a gap of at least a week from the 1 month time point, thus improving the chance of detecting any time effects present. It was important to ensure that the choice of time interval was not responsible for the results obtained. We therefore also re-tested all clinical correlations using 2 alternative measures for this period: each patient’s first ever physiological assessment (ie a single value), and a mean value across 4 weeks. The results obtained were very similar, providing reassurance that the precise choice of time interval did not determine the results. Group means were calculated in each parameter for this acute value and for all subsequent time points.

We tested whether physiological parameters covaried with ARAT scores across the acute period. In order to do this it was important to eliminate baseline differences across the group, such that any observed correlations would reflect longitudinal covariance rather than cross-sectional correlation. For each patient, mean values were first determined for

both the physiological parameter in question and the ARAT score during the acute period. Individual time points were then expressed relative to the patient's mean value: (individual value / mean value). This process allowed data points from different patients to be combined in order to test for longitudinal correlations across the acute period.

All data sets were tested for a normal distribution, using the Kolmogorov-Smirnoff test: of 58 data sets tested, 6 deviated from normal. In order to avoid inconsistent treatment of the data at different time points we have therefore used parametric statistics throughout. Changes with time in each physiological parameter in each hemisphere were examined using a repeated measures analysis of variance (ANOVA) with the factors Time and Hemisphere. Differences between the unaffected and affected hemispheres were tested using paired t-tests, while differences from the healthy control group were tested using unpaired t-tests, Bonferroni-corrected for multiple comparisons. Correlations were tested using linear regression analysis between physiological and behavioural measures. These values are not corrected for multiple comparisons but all correlation coefficients and corresponding P values are supplied below (see section 3.4.3).

3.3 Results

3.3.1 Patient group and behavioural results

Clinical details of the patients are given in Table 3.1. The group contained 6 males and 4 females, aged between 19 and 82 (58.0 ± 16.2 years mean \pm SD). One patient was unavailable for assessment at 3 months after the stroke and one other at 6 months. The

control group consisted of ten healthy volunteers, 7 male and 3 female, aged between 23 and 80 (56.2 ± 15.4 years).

Table 3.1. Patient characteristics

Initial severity describes the weakest upper limb muscle group (MRC scale) at the time of maximum weakness. M = Male; F = Female; L = Left; R = Right; M1 = primary motor cortex; MCA = Middle Cerebral Artery; PCA = Posterior Cerebral Artery; PUD = Peptic Ulcer Disease; AF = Atrial Fibrillation; IHD = Ischaemic Heart Disease; CAH = Chronic Active Hepatitis; IDDM = Insulin-Dependent Diabetes Mellitus; DVT = Deep Vein Thrombosis; NIDDM = Non Insulin-Dependent Diabetes Mellitus; COPD = Chronic Obstructive Pulmonary Disease.

Table 3.1

Patient	Age (years)	Sex	Handed-ness	Affected Arm	Lesion site	1 st Assessed (days post)	Initial Severity (MRC)	Initial Barthel Score	Previous Medical History	Medication
1	67	M	R	L	R MCA	8	2	10	Hypertension, PUD Bladder surgery	Clopidogrel, Lisinopril Propranolol, Amlodipine
2	82	F	R	L	R Striato-Capsular	19	3	13	AF, Thyrotoxicosis IHD	Warfarin, Atenolol Thyroxine, Ramipril, Digoxin
3	67	F	R	L	Isolated R M1	15	3	20	Hypertension Asthma, CAH	Aspirin, Amlodipine Azathioprine Prednisolone
4	50	M	R	R	L Striato-Capsular	10	4	20	AF, Hypertension Liver Transplant Hepatitis C, IDDM	Warfarin, Insulin Mycophenilate Thiamine, Citalopram
5	19	F	L	L	R MCA	7	0	15	DVT, Post-infectious Arthritis	Aspirin, Simvastatin Granisetron
6	65	M	R	L	R MCA	8	4	13	IHD, PUD, Head Injury (no surgery)	Clopidogrel, Atenolol Atorvastatin, Imdur Codeine Phosphate
7	59	M	R	L	R MCA (sparing cortex)	10	1	5	Appendicectomy Skin neoplasm	Aspirin, Atorvastatin Omeprazole
8	57	M	R	L	R MCA (sparing cortex)	11	3	18	Nil	Aspirin, Atorvastatin Temazepam (nocte)
9	58	M	R	R	L pons	6	1	10	Hypertension NIDDM, Gout	Aspirin, Simvastatin, Diltiazem, Gliclazide Metformin, Omeprazole
10	55	F	R	L	R Striato-Capsular	7	4	20	Hypertension, COPD Raised cholesterol Mild depression Paroxysmal hemiscranial	Aspirin, Ezetimide Candesartan, Pizotifen Sertraline, Omeprazole Salbutamol (inhaler)

Upper limb function was assessed by the ARAT and NHPT. Fig 3.2A shows results from every assessment within the first 3 months while Figure 3.2B shows data at the principal time points. There were significant improvements in both scores by 1 month (paired t-tests of initial assessment vs 1 month: ARAT $P=0.007$, NHPT 0.042), but no further significant changes (1 month vs 3 months, 3 months vs 6 months). Floor and ceiling effects were noted with the NHPT and ARAT respectively. Thus heterogeneity was better represented by the ARAT in the first month after stroke, and by the NHPT at 3-6 months.

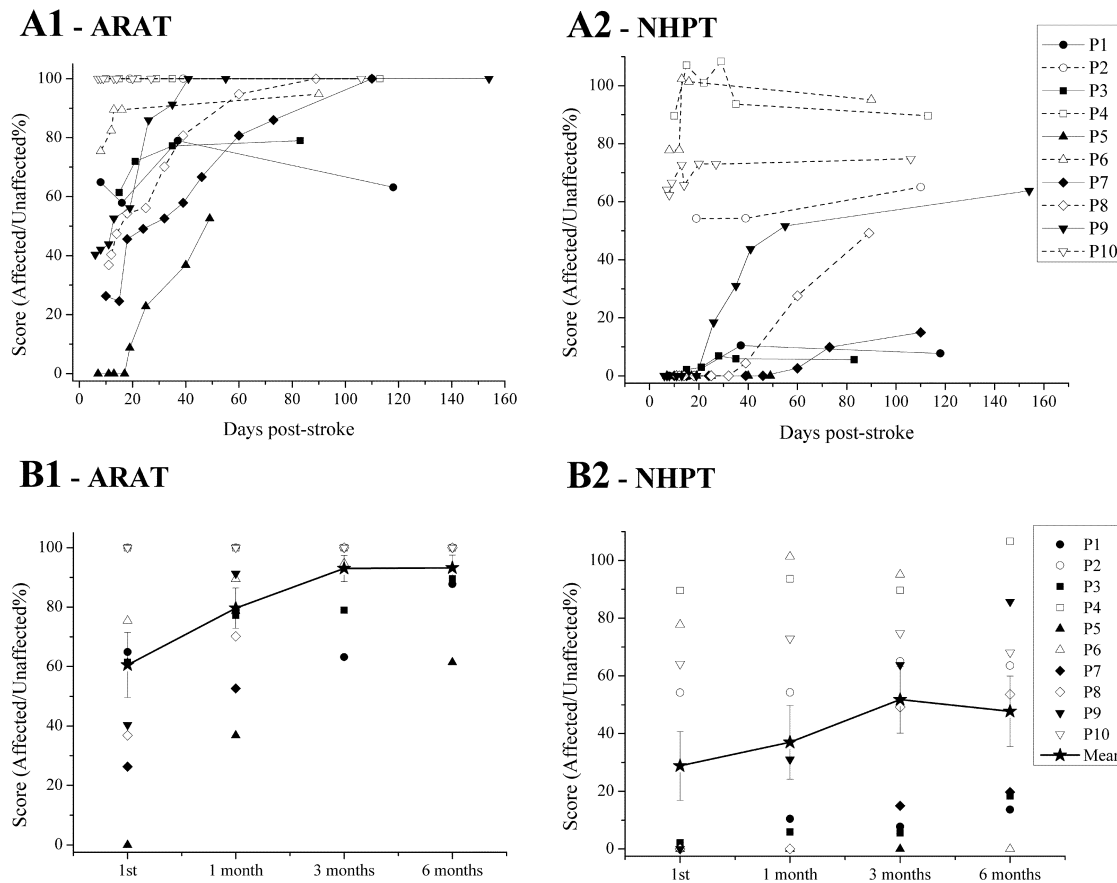


Figure 3.2. Upper limb clinical scores

Scores in the Action Research Arm Test (ARAT) and Nine Hole Peg Test (NHPT) are shown as performance in the affected limb as a percentage of that in the intact limb.

- A.** Individual scores in the ARAT (**A1**) and NHPT (**A2**) are shown for every assessment performed in the first (approximately) 3 months.
- B.** Scores are displayed for each patient at the principal time points, along with group means. Both ARAT (**B1**) and NHPT (**B2**) were significantly improved by 1 month (paired t-tests $P < 0.05$ vs initial assessment) and changes beyond this point were not significant. A number of patients had NHPT scores of zero (or near zero) at the first assessment, while many had a maximum ARAT score by 3 months - thus the ARAT is more informative of the two tests in the early period while the NHPT becomes more sensitive later on.

Compared to initial assessment, improvements were significant by 1 month after stroke in Motricity Index, NIHSS, 10 metre walk and Barthel Index (paired t-tests: P values, 0.035, 0.002, 0.031 and 0.033 respectively) and almost significant in the modified Rankin score ($P = 0.051$) (Figure 3.3). From 1 month to 3 months there were further significant improvements in 10 metre walk ($P = 0.027$) and Barthel index ($P = 0.0499$) but in no other tests. There were no further significant changes beyond 3 months.

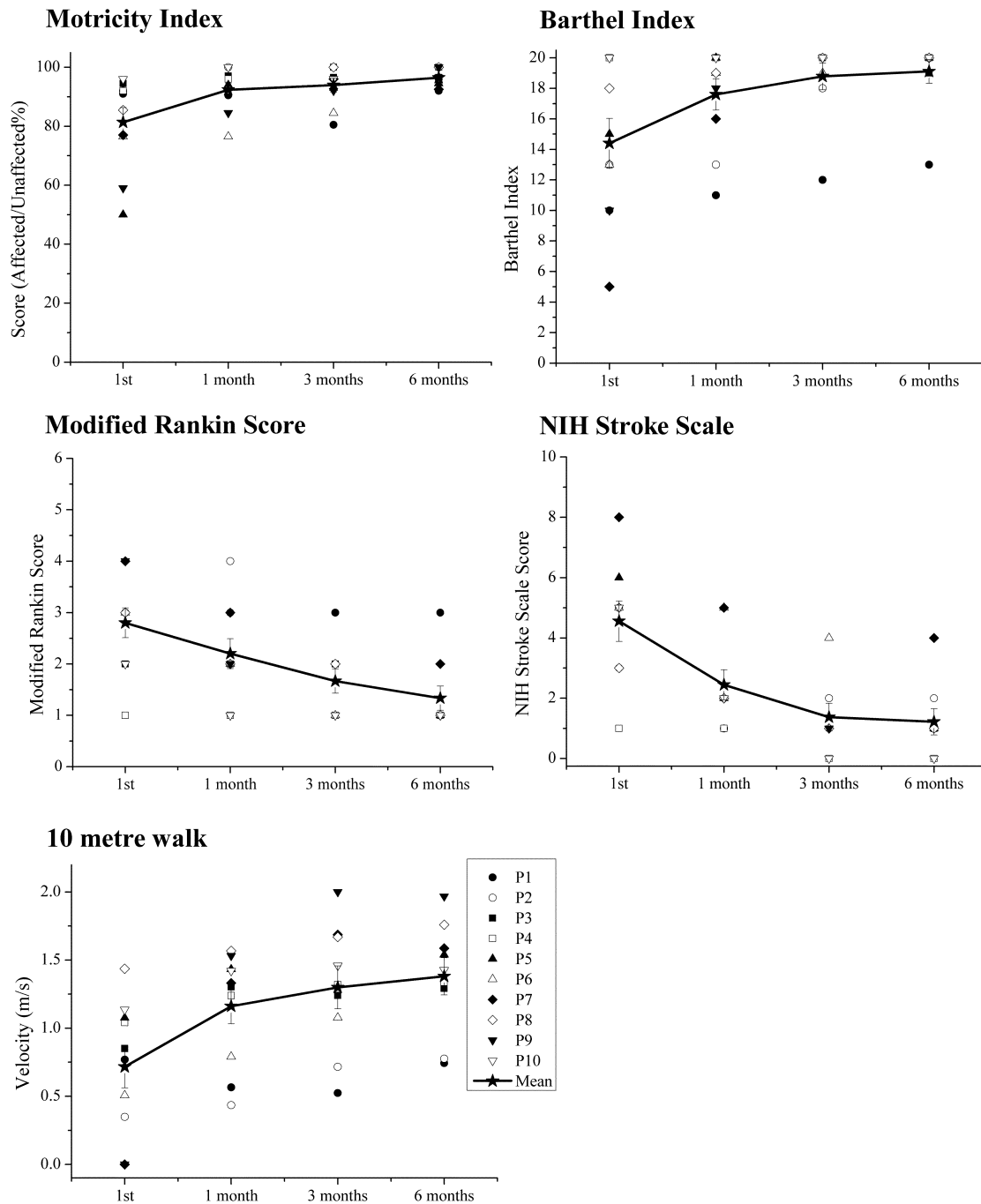


Figure 3.3. Global Clinical scores

Patients made significant gains in all scores other than the Modified Rankin score. Please see text for details.

Most recovery of upper limb function was thus observed in the first month, with some additional improvement in global scores up to 3 months.

3.3.2 Measures of corticospinal tract excitability

3.3.2.1 Motor thresholds

Figure 3.4A shows an illustration of all data collected within the first 40 days, while Figure 3.4B shows group means at each time point for resting and active motor thresholds. The value for the acute period represents a mean, within each patient, of all assessments made within 3 weeks of the stroke (see 3.2.4). Absolute values at each time point are given in Table 3.2.

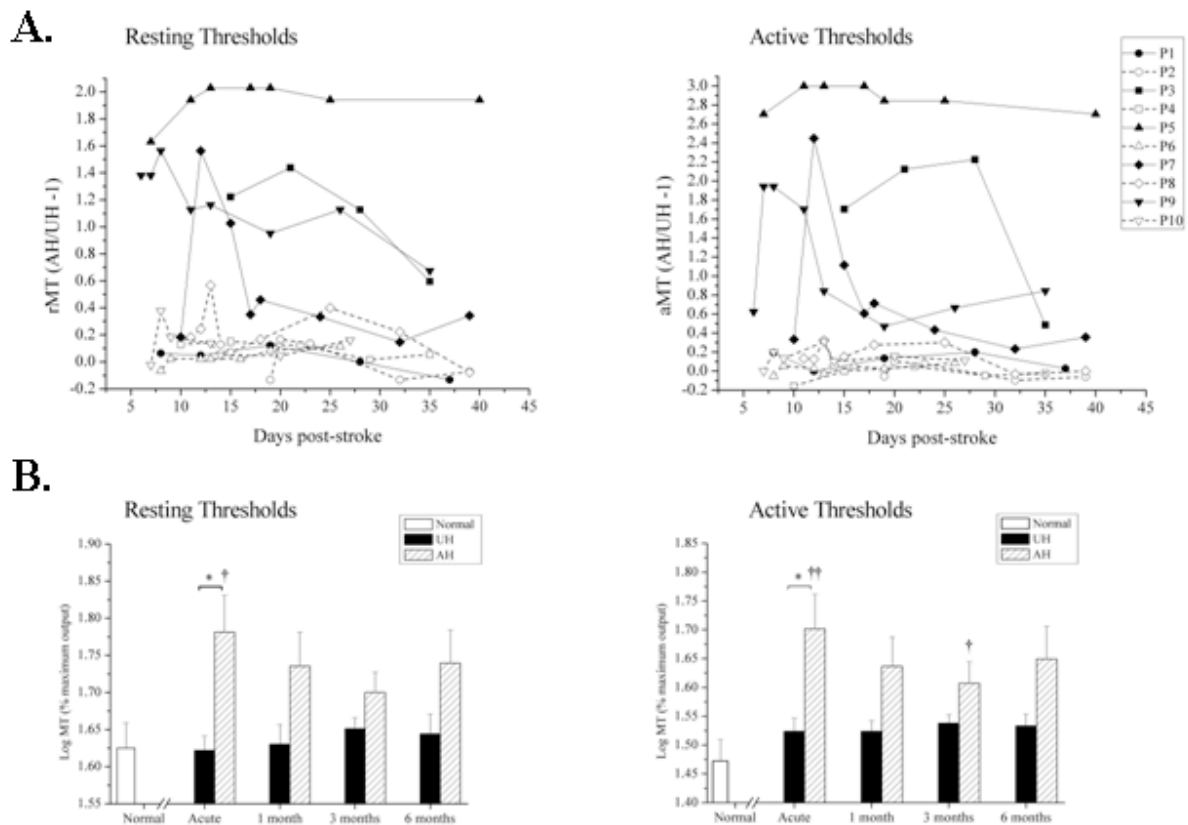


Figure 3.4. Motor thresholds

A. Motor thresholds are shown for each patient over the first 40 days. Resting (rMT) and active (aMT) motor thresholds are shown with thresholds in the affected hemisphere (AH) normalised to those in the unaffected hemisphere (UH), in order to reduce the impact of between-subject variability. Thus, a number greater than zero implies that thresholds were raised in the AH with respect to the UH. There is considerable within-subject variability of motor thresholds during this early period, in particular during the first 3 weeks.

B. Group means values for motor thresholds are shown at the principal time points, shown here as the logarithm of percentage of maximum stimulator output. The value described as acute has been determined in each patient as the mean of all values within the first 3 weeks. Time has differing effects on rMT in the 2 hemispheres – thresholds are initially raised in the AH and subsequently reduce but do not significantly change in the UH. For aMT there is a trend reduction in the AH from the acute period to 3 months but no Time x Hemisphere interaction and no Time effect across all 4 time points (see text for ANOVA details). Both rMT and aMT are significantly higher in the AH than the UH during the Acute period (paired t-tests: * $P < 0.05$) but this difference is not significant later. rMT values in the AH are raised compared to the healthy group only during the acute period, while aMT values are also raised at 3 months (unpaired t-tests: † $P < 0.05$, corrected for multiple comparisons).

	Acute period	1 month	3 months	6 months
Resting thresholds				
UH	42.3 ± 2.0 %	43.4 ± 2.6 %	45.0 ± 1.5 %	44.8 ± 2.9 %
AH	64.3 ± 7.8 %	57.4 ± 6.6 %	51.0 ± 3.6 %	57.4 ± 6.5 %
Active thresholds				
UH	33.9 ± 1.9 %	33.7 ± 1.5 %	34.7 ± 1.1 %	34.4 ± 1.7 %
AH	55.2 ± 8.5 %	46.5 ± 6.7 %	41.8 ± 4.1 %	48.2 ± 7.7 %

Table 3.2. Motor Thresholds

Group means (\pm S.E.) are shown at each time point for the resting and active motor thresholds, as percentage of maximum stimulator output (UH = unaffected hemisphere; AH = affected hemisphere).

Early variability

There was considerable within- and between-subject variability in motor thresholds during the first three weeks after stroke. We examined the combined data from this early period for longitudinal correlations with clinical scores: the observed variability did not reflect fluctuations in clinical scores (combined correlations with ARAT scores for the first three weeks: rMT $r=-0.025$ (ns); aMT $r=-0.011$ (ns)). This suggests that such physiological fluctuations are not related in a simple way to early changes in clinical status, and must be explained in another way.

The effect of time on resting thresholds was different in the 2 hemispheres

For resting thresholds there was a significant Time x Hemisphere interaction (2-way ANOVA: $F_{3, 21}=8.24$; $P=0.022$,) that was due to the fact that thresholds decreased in the AH (1-way ANOVA: $F_{3, 21}=7.84$; $P=0.025$) but did not change in the UH (1-way

ANOVA: $F_{3,21}=1.57$; $P=0.307$). Such a reduction in resting thresholds implies that there was some recovery of corticospinal excitability in the AH during this period. However, the same was not true of active thresholds (aMT), for which there was no significant difference in the effects of time on the 2 hemispheres (2-way ANOVA: Time x Hemisphere $F_{3,21}=3.41$; $P=0.110$. Trend effect of Hemisphere $F_{1,7}=4.06$; $P=0.084$. No effect of Time).

Motor thresholds were raised in the affected hemisphere during the acute period

Both rMT and aMT were significantly higher in the AH than the UH during the acute period (paired t-tests: rMT $P=0.017$, aMT $P=0.030$) but this difference was not significant at later time points. Comparison with the healthy control group revealed that rMT was significantly raised during the acute period but not later (un-paired t-tests, values Bonferroni corrected for multiple comparisons: acute $P=0.038$, 1 month $P=0.140$, 3 months $P=0.160$, 6 months $P=0.115$), while aMT was significantly raised during the acute period and at 3 months (acute $P=0.015$, 1 month $P=0.051$, 3 months $P=0.039$, 6 months $P=0.058$). Corticospinal excitability as assessed by motor thresholds was thus impaired during the acute period.

We observed an early rise in active motor thresholds

Close inspection of the earliest time points (Figure 3.4A above) gives the impression that, contrary to the overall trend for thresholds to reduce with time, there was an initial increase over the first few assessments. In order to address this possibility, the first 3 measurements of motor thresholds were analysed separately (Figure 3.5).

Active Thresholds: 1st 3 assessments

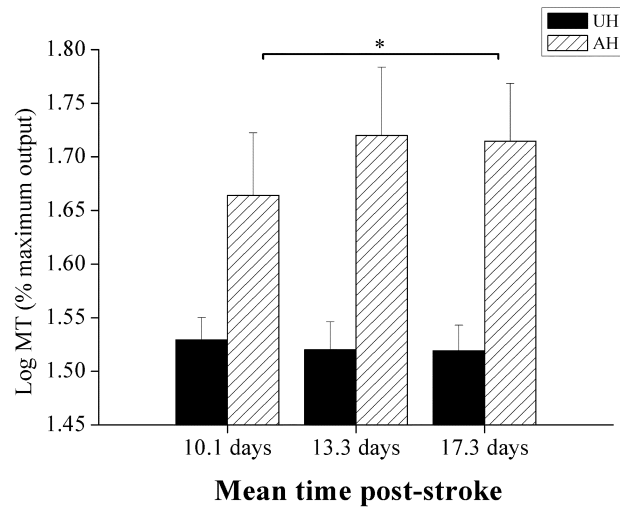


Figure 3.5. Early rise in active motor thresholds

Group means for the first 3 measurements of active motor thresholds (aMT) in each patient are shown. There was a significant increase in aMT in the AH between the first and third measurements (paired t-test: * $P=0.009$ – see text for ANOVA details). Thus an increase in aMT in this early period is followed by a reduction in thresholds between months 1 and 3 (Fig 3.4B).

There was a small variation in the time of the earliest measurements between patients, with the mean number of days after stroke being 10.1 ± 1.3 (mean \pm S.E.), 13.3 ± 1.4 and 17.3 ± 1.9 respectively. There was a significant Time x Hemisphere interaction (2-way ANOVA: $F_{2,18}=13.02$; $P=0.003$). This was due to the fact that there was a significant increase in aMT from the 1st to 3rd assessment in the AH (paired t-test, $P=0.009$), but not in the UH. Corticospinal excitability as measured by aMT thus declined further at this

early stage, contrary to the longer-term pattern of improvement. There was no equivalent effect for rMT.

3.3.2.2 MEP Recruitment Curves

An illustration of all data collected in the first 40 days is shown in Figure 3.6A, while Figure 3.6B shows group means at each time point.

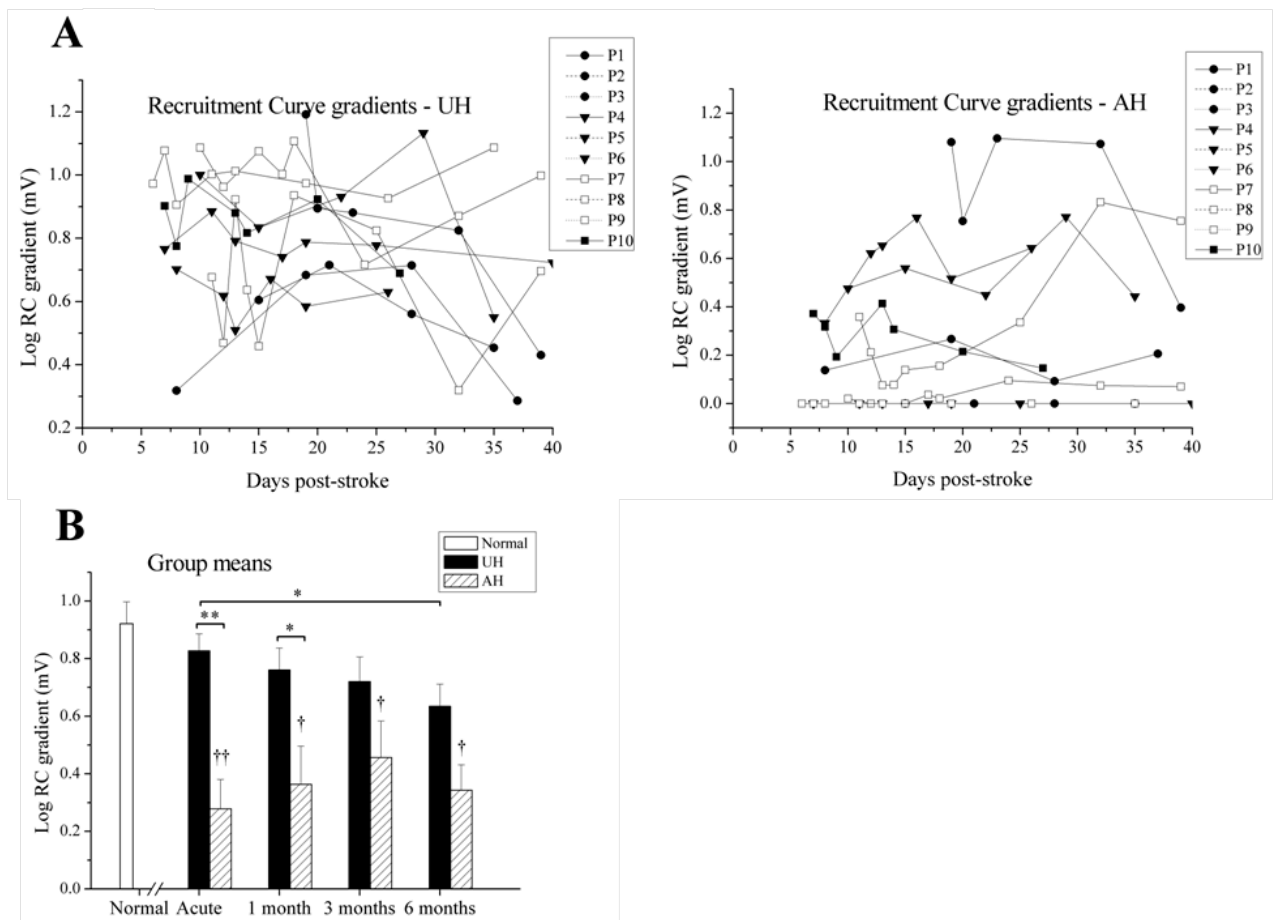


Figure 3.6. Recruitment Curve gradients

A. Recruitment Curve (RC) gradients in either hemisphere (UH and AH) are shown for each patient over the first 40 days. As for motor thresholds, most patients show considerable variability of this measure in the early period (except for those with poor responses in the AH).

B. Group means are shown for Recruitment Curve (RC) gradients in either hemisphere at the principal time points. There is a significant decrease in excitability in the UH from the acute period to 6 months (paired t-test: * $P < 0.05$) and a trend increase in the AH ($P = 0.059$). Excitability in the AH is significantly reduced with respect to the UH at the first 2 time points (paired t-tests: * $P < 0.05$, ** $P < 0.01$) but this difference is not significant at later time points. Compared to the healthy control group, RC gradients are significantly reduced in the AH at all time points (un-paired t-tests: †† $P < 0.01$, † $P < 0.05$, corrected for multiple comparisons) while those in the UH are not significantly different from normal. Please see text for ANOVA details.

Early variability

Recruitment curve (RC) gradients in the UH and AH over the first 3 weeks revealed considerable variation between patients with some showing substantial recovery of excitability, while in others gradients remained low. This early variability did not reflect fluctuations in clinical scores (combined correlations with ARAT scores during the acute period: UH $r = 0.023$ (ns); AH $r = 0.059$ (ns)). This supports the idea that fluctuations in corticospinal excitability do not relate simply to clinical variability during this period.

The effect of time on RC gradients was different in the 2 hemispheres

There was a significant Time x Hemisphere interaction (2-way ANOVA: $F_{3, 21} = 9.08$; $P = 0.018$. Significant effect of Hemisphere $F_{1, 7} = 7.05$; $P = 0.033$), suggesting that this measure of corticospinal excitability changes in opposite directions with time in the 2 hemispheres. Follow-up one way ANOVAs were performed separately for each hemisphere: although there was no significant change across all 4 time points in either

hemisphere, there is a tendency for the UH gradients to decline (decreasing UH excitability) and the AH gradients to increase (increasing AH excitability). When the acute period was directly compared to 6 months these effects were more marked, with a stronger Time x Hemisphere interaction (2-way ANOVA: $F_{1,8}=15.56$; $P=0.004$. Significant effect of Hemisphere $F_{1,8}=14.64$; $P=0.005$), a significant excitability reduction in the UH (paired t-test, $P=0.042$) and a trend increase in the AH ($P=0.059$).

RC gradients in the affected hemisphere remained reduced compared to normal

RC gradients were significantly lower in the AH than the UH during the acute period and at 1 month (paired t-tests, $P=0.001$ and $P=0.035$ respectively) but this difference was not significant later. Comparison with the healthy control group revealed that excitability was reduced in the AH at all time points (un-paired t-tests, values Bonferroni corrected for multiple comparisons: acute $P<0.001$, 1 month $P=0.011$, 3 months $P=0.033$, 6 months $P=0.001$). Despite the trend for excitability in the AH to increase across the period studied, it did not therefore recover to normal levels. RC gradients in the UH did not differ from the control group.

3.3.3 Measures of intracortical excitability

An illustration of all data collected in the first 40 days for Short Interval Intracortical Inhibition (SICI), Intracortical Facilitation (ICF) and Long Interval Intracortical Inhibition (LICI) is shown in Figure 3.7, while Figure 3.8 shows group means at each time point.

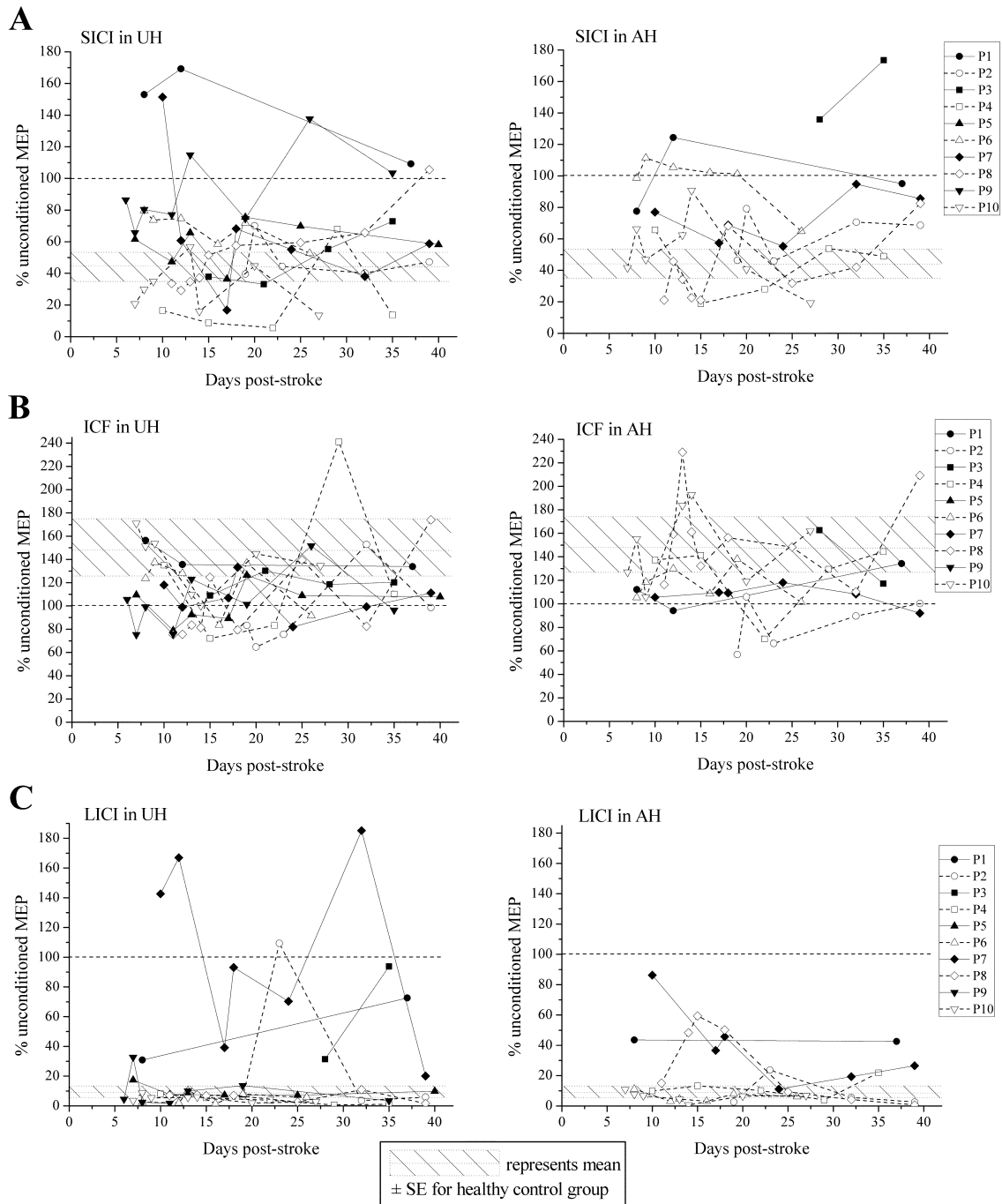


Figure 3.7. Intracortical Excitability – the first 40 days

The values of 3 measures of intracortical excitability are shown in each hemisphere and at every assessment, as measured by paired pulse TMS (SICI = Short Interval Intracortical Inhibition, ICF = Intracortical Facilitation, LICI = Long Interval Intracortical Inhibition). The value

shown in each case represents the percentage change of the response to a test stimulus in the presence of a conditioning stimulus. Values for the healthy control group are shown as a shaded area (mean \pm standard error). As for the measures of corticospinal excitability, variability is great in the early period.

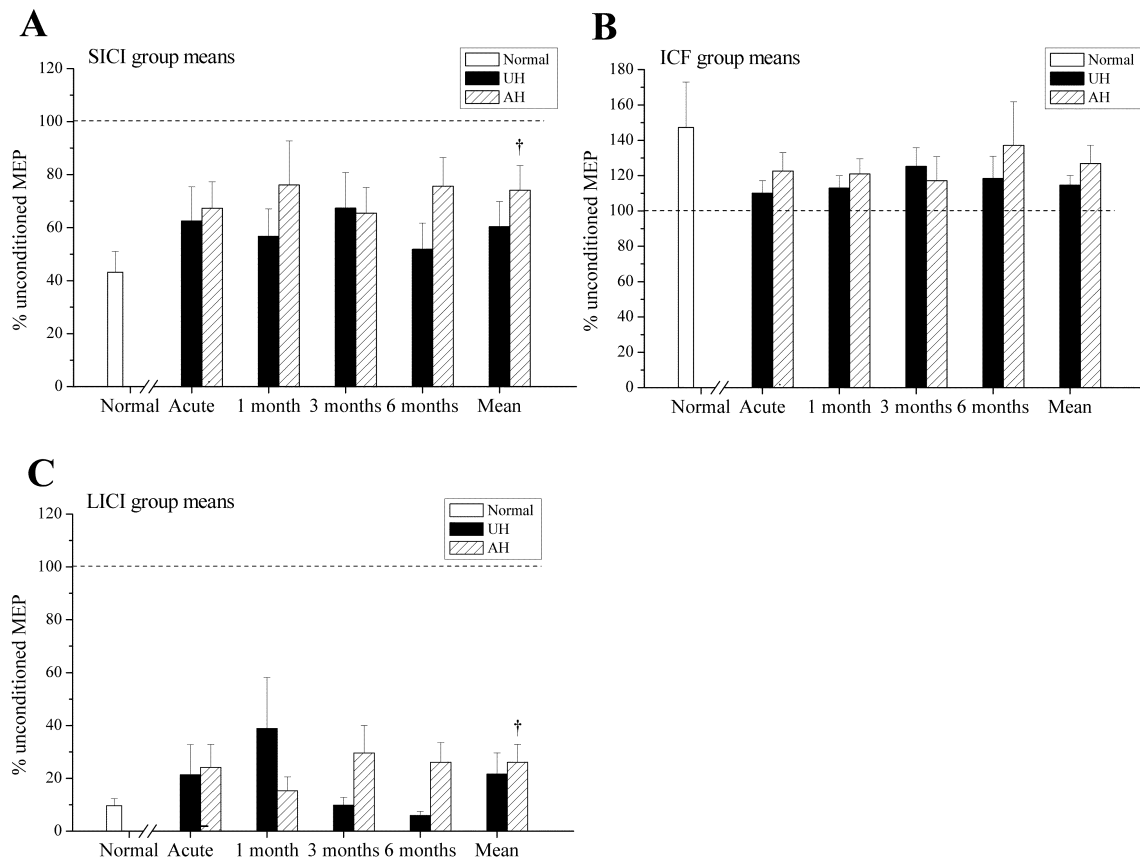


Figure 3.8. Intracortical Excitability – main time points

Mean values at the principal time points are shown for 3 measures of intracortical excitability: Short Interval Intracortical Inhibition (SICI – **A.**), Intracortical Facilitation (ICF – **B.**) and Long Interval Intracortical Inhibition (LICI – **C.**). None of these values were significantly different from the healthy control group at individual time points (unpaired t-tests, values corrected for multiple comparisons). No parameter showed a significant Time x Hemisphere interaction. A

mean value across all time points was thus calculated in each patient (and each parameter and hemisphere). This mean value is significantly raised compared to the normal group in the Affected Hemisphere for SICI and LICI (un-paired t-tests: * $P < 0.05$). This suggests that these forms of intracortical inhibition are weak in the affected hemisphere (ie increased excitability to paired pulse stimuli). The values for SICI and LICI also appear raised in the unaffected hemisphere, but are not significantly different from the healthy control group.

Early variability

As with other data collected in this period, there is substantial variation both within and between subjects for the AH and the UH. There is no clear trend to normalisation or worsening. Variability during the three weeks after stroke did not reflect fluctuations in clinical scores for any of the intracortical excitability parameters in either hemisphere (combined correlations with ARAT scores during the acute period: r values for the UH between -0.099 and 0.352 (ns); r values for the AH between -0.123 and 0.216 (ns)). As described above for corticospinal excitability, it therefore appears that fluctuations in intracortical excitability likewise do not relate in a simple manner to clinical variability during this early period.

There were no time effects. Inhibition (SICI and LICI) was weak in the affected hemisphere

No clear trends were observed in these parameters up to 6 months. The 2-way Time x Hemisphere ANOVAs for each parameter showed no significant main or interaction effects. In view of the lack of Time effects, a mean value for each parameter across all

assessments was calculated for every patient and in each hemisphere (shown in Figure 3.8) to allow comparison with values from the healthy control subjects. SICI and LICI (but not ICF) were significantly reduced in the affected hemisphere when compared to the healthy control group (unpaired t-tests: SICI $P=0.015$, LICI $P=0.029$), but were not significantly reduced in the unaffected hemisphere (SICI $P=0.157$, LICI $P=0.123$). Mean values for SICI were $74.1 \pm 9.3\%$ of MEP amplitude after a single pulse (patients, affected hemisphere), $60.4 \pm 9.5\%$ (patients, unaffected hemisphere) and $44.0 \pm 5.6\%$ (healthy volunteers). Mean values for LICI were $26.1 \pm 6.8\%$ of MEP amplitude after a single pulse (patients, affected hemisphere), $21.6 \pm 8.0\%$ (patients, unaffected hemisphere) and $7.8 \pm 2.1\%$ (healthy volunteers). The significantly larger conditioned MEPs (relative to unconditioned) in the patient group imply that these 2 forms of inhibition are weak in the affected hemisphere.

3.3.4 Relating physiological measures to clinical performance

At each of the principal time points, correlations were examined between the TMS measures and upper limb clinical scores (ARAT and NHPT). Two examples of such plots are shown in Figure 3.9 (Individual correlation plots can be seen in Supplementary figures 3.1-3.5, while the complete set of correlation coefficients is given in Table 3.3). A graphical summary of changes in these correlations over time is displayed in Figure 3.10. Because of respective floor and ceiling effects for early NHPT scores (first month) and later ARAT scores (3 months and beyond), acute period values shown in Figure 3.10 relate to correlations with ARAT scores while later values relate to correlations with NHPT scores.

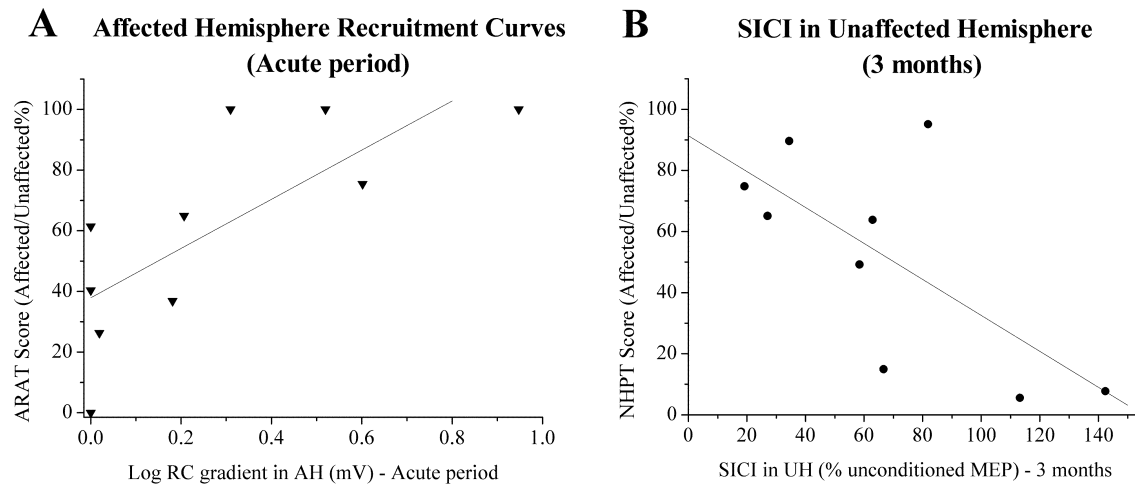


Figure 3.9. Correlations with clinical status – 2 examples

The relationships between measures of clinical performance and 2 physiological parameters are shown, each point representing a patient.

A. There is a significant positive correlation between affected hemisphere Recruitment Curve (RC) gradients in the Acute period and Action Research Test (ARAT) scores at initial assessment ($r=0.754$, $P=0.006$). **B.** Short Interval Intracortical Inhibition (SICI) in the unaffected hemisphere is negatively correlated with Nine Hole Peg Test (NHPT) scores at 3 months, such that weaker inhibition (ie increased excitability to paired pulse TMS) is associated with poor clinical status ($r=-0.686$, $P=0.021$).

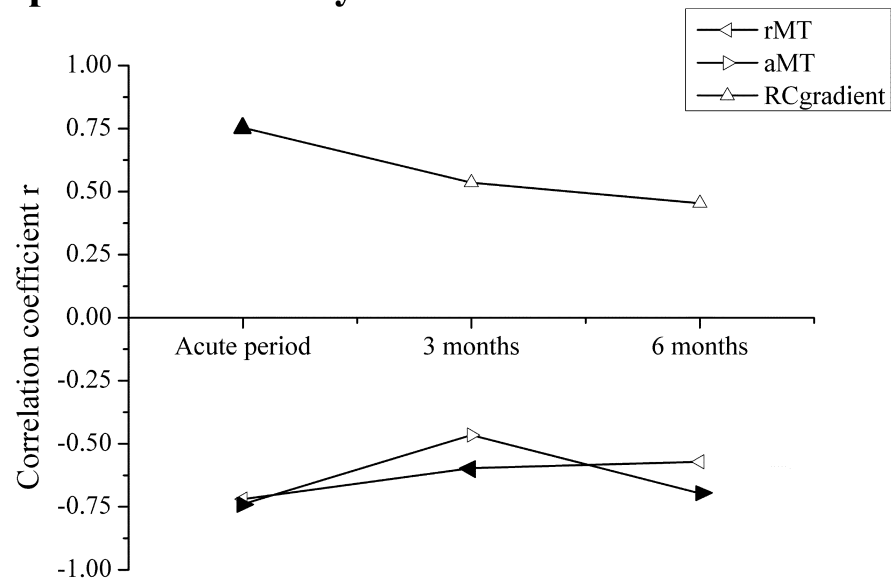
Table 3.3

		Acute ARAT		NHPT		3 Months ARAT		NHPT		6 months ARAT		NHPT	
rMT		-0.720	(0.0094)	-0.620	(0.0281)	-0.292	(0.2236)	-0.597	(0.0449)	-0.837	(0.0025)	-0.572	(0.0539)
aMT		-0.740	(0.0073)	-0.657	(0.0196)	-0.090	(0.4061)	-0.466	(0.1032)	-0.837	(0.0025)	-0.696	(0.0186)
RC Gradient	UH	0.077	(0.4162)	0.148	(0.3416)	0.403	(0.1412)	0.259	(0.2509)	-0.150	(0.3502)	-0.393	(0.1477)
	AH	0.754	(0.0059)	0.774	(0.0043)	0.449	(0.1128)	0.535	(0.0687)	0.545	(0.0648)	0.454	(0.1100)
SICI	UH	-0.224	(0.2667)	-0.437	(0.1033)	-0.895	(0.0006)	-0.686	(0.0206)	-0.078	(0.4212)	-0.220	(0.2845)
	AH	-0.020	(0.4830)	-0.038	(0.4680)	-0.526	(0.0728)	-0.550	(0.0627)	-0.418	(0.1314)	-0.134	(0.3758)
ICF	UH	0.160	(0.3298)	0.030	(0.4670)	-0.613	(0.0395)	-0.693	(0.0192)	0.179	(0.3228)	0.464	(0.1234)
	AH	-0.096	(0.4186)	0.098	(0.4174)	-0.237	(0.2695)	-0.329	(0.1939)	-0.287	(0.2269)	0.077	(0.4350)
LICI	UH	-0.377	(0.1589)	-0.415	(0.1334)	-0.845	(0.0041)	-0.737	(0.0117)	0.258	(0.2687)	0.412	(0.1354)
	AH	-0.908	(0.0024)	-0.907	(0.0024)	-0.656	(0.0387)	-0.636	(0.0327)	-0.225	(0.2958)	-0.315	(0.2240)

Table 3.3. Correlation coefficients

Correlation coefficients are shown for the plots of each physiological parameter against the clinical scores shown (rMT and aMT are resting and active motor thresholds respectively; RC = Recruitment Curve; SICI = Short Interval Intracortical Inhibition; ICF = Intracortical Facilitation; LICI = Long Interval Intracortical Inhibition; ARAT = Action Research Arm Test; NHPT = Nine Hole Peg Test). The first 3 parameters are plotted as semi-log plots, as shown in Figure 3.4B. P values are shown in brackets.

A - Corticospinal Excitability



B - Intracortical Excitability

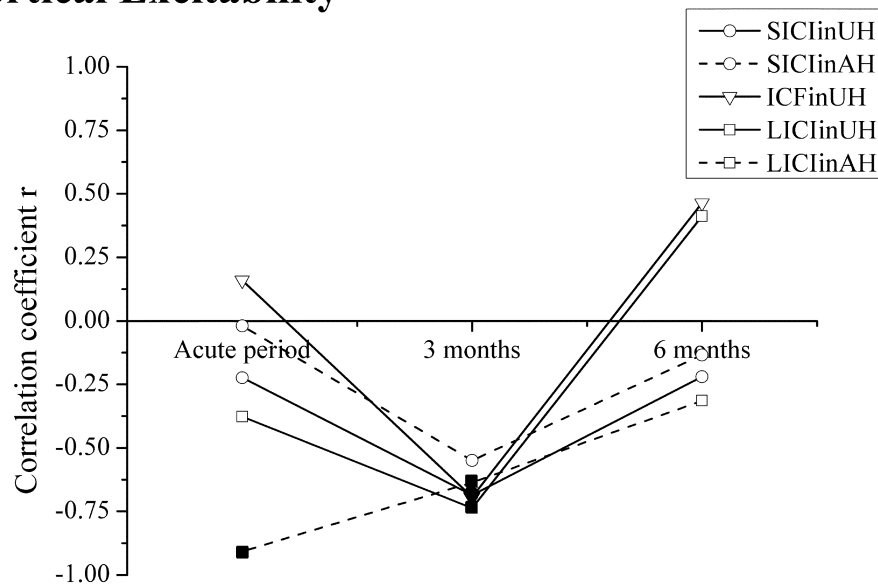


Figure 3.10. Correlations with clinical status – changes with time

Correlation coefficients between the physiological parameters measured and clinical outcome scores are presented for the 3 phases of stroke recovery studied. Significant correlations are denoted by filled symbols ($P < 0.05$). Because of the respective floor and ceiling effects seen in Nine Hole Peg Test (NHPT) and Action Research Arm Test (ARAT) scores in the early and late stages, the correlations in the

acute period are with ARAT scores and those at later time points are with NHPT scores. The complete correlation coefficients are given in Table 3.3, and the plots can be seen in Supplementary Figures 3.1 to 3.5.

Different changes in clinical correlations with time were observed for measures of corticospinal and intracortical excitability. In the acute period there were strong relationships between clinical scores and all 3 measures of corticospinal excitability in the AH (rMT, aMT and RC gradients): small RC gradients or raised motor thresholds were associated with poor clinical status. At 3 and 6 months this relationship had weakened for RC gradients and was slightly weaker for motor thresholds. By contrast, as shown in Figure 3.10B, measures of intracortical excitability (SICI, ICF and LICI), apart from ICF in the AH which was not correlated at any stage (not shown), behaved very differently. Relationships to clinical scores were weak in the acute period (except for LICI in the AH) but strong at 3 months: at this time raised intracortical excitability to paired pulse TMS (weak inhibition or strong facilitation) was associated with poor clinical status. At 3 months, all the intracortical excitability measures shown in Figure 3.10B correlated significantly with NHPT scores (except for SICI in the AH: $P = 0.063$). This relationship had disappeared at 6 months.

To summarise, correlations between clinical status and measures of corticospinal or intracortical excitability changed over time after stroke: correlations with corticospinal excitability were highest in the acute stage whereas correlations with intracortical excitability were initially poor, but then increased strongly at 3 months.

3.4 Discussion

The present data provides a longitudinal picture of bilateral cortical physiology in this patient group over a six month period after stroke. The results confirm several previously described neurophysiological abnormalities, but additionally describe their evolution.

We also report changes in LICI after stroke for the first time. Most importantly, we have been able to correlate physiological parameters with clinical function at several time points. The results suggest that in the period following stroke corticospinal and intracortical excitability evolve differently in their relationship to motor function. We argue that these changes in clinical correlation over the 6 month period reflect shifts in how a motor output to the paretic hand is generated.

3.4.1 The patient group

Patients were recruited consecutively from an acute stroke unit and were heterogeneous with regard to impairment and lesion site. A relatively wide range of impairment reduces the statistical power of comparisons with healthy controls and may therefore give false negatives when testing for abnormalities. However this range has the complementary advantage of allowing correlations with functional status to be examined, providing insight into the clinical relevance of the parameters tested. It is also worth noting that there was a wide age range in the patient group, with a mean patient age (58 years) that is somewhat younger than mean age at stroke onset in the wider population. Although this is a potential source of physiological variance, it would not be expected to contribute to the changes observed with time.

While all patients studied had a single infarction involving the corticospinal tract, the primary motor cortex (M1) was involved in four out of ten, the other six having purely subcortical infarctions. The inclusion of patients with cortical involvement raises the possibility that disruption of transcallosal neural populations may have altered the observed pathophysiology in some patients. While Liepert et al (2005) found differences in intracortical inhibition depending on whether M1 was involved in chronic stroke patients, other studies with mixed groups have observed no such distinction in the acute (Manganotti et al 2002) or chronic (Cicinelli et al 2003) periods. It is difficult to know whether cortical involvement affected the changing relationships that we have described without studying two larger groups. However, it is by no means clear that patients classified as having ‘cortical infarcts’, on the basis of damage involving M1, can be regarded as homogenous. As well as the involvement or otherwise of transcallosal fibres, such a categorisation would have to make allowance for the extent of damage to ipsilesional non-primary cortical areas and their topographically distinct underlying white matter pathways (Newton et al 2006) as well as deeper structures in the basal ganglia and thalamus. A simple distinction between cortical and subcortical may therefore not be useful in this context. Even among a homogeneous patient group with only subcortical infarctions the pattern of reorganisation is determined crucially by the degree of corticospinal disruption (Ward et al 2006, 2007). A similar observation has been made using a diffusion tensor imaging measure of corticofugal fibre tract disruption, in a homogeneous group, and relating this measure to motor function (Stinear et al 2007). We observed such a relationship here, between intracortical excitability measures and clinical

function, in a more heterogeneous group. It may be therefore that impaired corticospinal output, rather than lesion location per se, is the primary determinant of altered excitability in the distributed cortical network. Further studies which are designed to test this hypothesis specifically are required.

It should be noted that three patients took neuroactive medications. Citalopram (P4) can increase SICI but only in subjects with the long/long polymorphism within the promoter region of the serotonin transporter gene, 5-HTTLPR (Eichhammer et al 2003) – such genetic information is unavailable for this patient. Temazepam (P8), a Benzodiazepine, can increase SICI (Ziemann et al 1996c) but was taken here in the evening – it has a short effect, reaching peak plasma concentration in 45 minutes (Wang & Devanne 2003), so is unlikely to affect SICI the following day. Sertraline (P10) can increase MEP amplitudes for up to 2 hours and reduce ICF for up to 6 hours. With testing performed in the early afternoon, it is unlikely that MEP amplitudes were affected but the possibility cannot be excluded that ICF was reduced. None of these medications were changed during the course of the study, however, so that any potential distortion of physiological measures should not explain the time effects observed.

3.4.2 Behavioural measures

Scores in all behavioural tests were significantly improved by one month (except for the modified Rankin score). The tests of upper limb function (ARAT and NHPT) did not show further significant change beyond this point, but differed as to when they most usefully described the clinical heterogeneity: the NHPT showed a floor effect during the

acute period, while the ARAT showed a ceiling effect by 3 months. This observation is consistent with previous reports of the respective characteristics of these tests (Wade 1992). The ARAT is partly dependent on proximal power and simple grasp movements, whereas the NHPT relies also on the ability to perform fractionated finger movements, to hold a peg between thumb and finger, and to pronate the wrist (in order to insert the peg). The differing time courses for improved performance of these two tests reflects recovery of proximal upper limb function before hand function, and is a reminder that in order to accurately describe clinical heterogeneity it is important to choose a behavioural test appropriate to the stage of recovery.

The upper limb scores were expressed relative to those obtained in the unaffected arm, in order to minimise the impact of any global factors on test performance. This is likely to be particularly important during the early weeks – crucial for this study – when patients are subject to fluctuations in a variety of systemic factors which may reduce effort globally. This approach has been used in a number of other publications in the field of stroke recovery (Ward et al 2003b; Murase et al 2004; Ward et al 2006; Talelli et al 2006). It is certainly true that the unaffected arm may perform sub-optimally on tests of dexterity, when compared to a healthy control group. Such deficits are seen chiefly in the context of left hemispheric cortical strokes, in association with the resulting cognitive deficit (Sunderland 2000), whereas all of the patients with cortical involvement in our study had infarctions on the right side. However, subtle deficits have recently also been reported ipsilateral to subcortical stroke (Noskin et al 2008; Nowak et al 2007).

3.4.3 TMS measurements in the acute period

TMS data from either hemisphere at multiple time points within the first month revealed great within-subject variability. Unexpectedly, there was no relationship between the observed physiological variations and motor performance in this period. The reason for this is unclear. It may be argued that fatigue in the early weeks after stroke could exert a fluctuating global influence on the parameters measured. Alternatively, changes may result from the developing effects of diaschisis, as the functional loss of the affected region exerts a distributed effect. Finally, fluctuations in cerebral perfusion in the post-stroke period, resulting from disturbed cerebrovascular auto-regulation, may well affect penumbral brain regions and thus affect physiological measures. Whatever the reason, our findings demonstrate that single physiological measurements made in the first 3 weeks after stroke have little clinical use on their own. In the present study we therefore averaged measurements acquired during the first 3 weeks in order to obtain a reliable measure of early post-stroke cortical physiology. This time interval was chosen in order to encompass the early weeks while remaining temporally distinct from the 1 month assessment. In order to ensure that the choice of time interval did not affect the observed pattern of results, we re-calculated the clinical correlations for this period using a 4 week period and also using only the first TMS session in each patient. Correlations for all the TMS parameters were remarkably similar using these alternative acute measures to those presented here, providing reassurance that the precise choice of time interval did not alter the results.

3.4.4 Corticospinal excitability

Motor thresholds

Both rMT and aMT were higher in the affected hemisphere than in the unaffected hemisphere (and higher than the healthy control group) during the acute period, while aMT was also raised at 3 months. Thresholds in the affected hemisphere reduced with time, significantly for the resting thresholds (rMT) but not for the active thresholds (aMT). Correlations with clinical scores persisted to 3 and 6 months in both measures, but were strongest in the acute period.

Raised motor thresholds in the affected hemisphere (AH) have been described in several reports (Catano et al 1996; Catano et al 1997a; Heald et al 1993; Traversa et al 2000; Nardone et al 2002). Here, the resting thresholds in the AH had largely normalised by 1 month, although active thresholds were still raised at 3 months. Examining the individual patient data (Supplementary Figure 3.2A) also suggests that a plateau had been reached by approximately a month, which is in agreement with previous work (Catano et al 1996; Traversa et al 2000). However, the degree to which thresholds normalised varied across the group, and power analyses suggest that abnormally raised active thresholds may have been detected with a larger group at 1 month and 6 months. Furthermore, there was still a significant correlation between aMT and functional status at 6 months, suggesting that this measure remained abnormal in more affected patients.

Reports of clinical correlations with motor thresholds have been variable, and have generally been described at single time points. In the acute period, an association of

raised motor thresholds with poor clinical status has been demonstrated at 1 day after stroke (Heald et al 1993) and within 14 days (Liepert et al 2005). It has also been suggested that motor thresholds in this period may have predictive value for eventual recovery (Heald et al 1993; Catano et al 1997b; Wittenberg et al 2007): negative correlations were likewise observed in the present study (not shown) between active thresholds in the acute period and clinical scores at 6 months ($P=0.058$), while the relationship was weaker for resting thresholds ($P=0.138$). A relationship between raised thresholds and poor function has also been reported when assessed in the chronic stage, both for rMT (Werhahn et al 2003) and aMT (Thickbroom et al 2002), although patients have also been described with normal function despite raised thresholds (Byrnes et al 2001). The present results suggest that the relationship may vary over time, becoming weaker by 6 months for rMT and to a lesser extent aMT. The difference between the clinical correlations observed for rMT and aMT in the chronic stage may be explained by increased spinal excitability (eg due to greater cortico-proprio-spinal drive - Mazevet et al 2003), which may reduce rMT. This compensation would not affect aMT, however, which is tested when motor neurons are already near their firing potential - thus aMT may more accurately reflect cortical excitability than rMT in the chronic stage (Talelli et al 2006).

The overall reduction in motor thresholds with time was preceded by an early increase in aMT (10 to 17 days). Since active motor thresholds reflect membrane excitability rather than synaptic activity an early increase could reflect ongoing structural or metabolic

consequences of the infarction, rather than altered input to M1, or other factors including decreasing membrane excitability at a spinal level due to immobility of the paretic limb.

Recruitment Curves

Recruitment curve gradients changed differently with time in the two hemispheres, tending to decrease in the unaffected hemisphere (UH) and increase in the affected hemisphere (AH). Gradients were smaller in the AH than in the UH during the acute period and at 1 month, and were reduced compared to the healthy control group at all time points studied. Gradients in the AH correlated strongly with clinical scores during the acute period but less so at later time points.

Most previous work studying corticospinal excitability after stroke has tested MEP amplitudes at a fixed stimulus intensity relative to motor threshold, during either relaxation (Cicinelli et al 1997; Traversa et al 2000; Trompetto et al 2000; Thickbroom et al 2002) or active contraction (Cicinelli et al 1997; D'Ohlaberriague et al 1997). This method is susceptible to errors resulting from small shifts in motor threshold.

Furthermore, the shape of the recruitment curve may change following stroke, such that a single stimulus intensity may reflect different points on this curve in different patients.

Here we have measured the Recruitment Curve (RC) gradient, determining MEP amplitudes at 4 stimulus intensities and calculating the slope of the resulting curve. The range of intensities makes this method less vulnerable to these 2 sources of error.

Persistent corticospinal hypoexcitability in the AH is consistent with previous results showing reduced MEP amplitudes after 3 months (Traversa et al 2000; Byrnes et al 2001; Thickbroom et al 2002; Delvaux et al 2003) and at 6 months (Pennisi et al 2002). A trend improvement with time is in keeping with a study during the subacute period by Cicinelli et al (1997): however, other studies at more than one time point have reported no increase (Delvaux et al 2003) or a significant increase (Traversa et al 2000). It is difficult to say what may account for the differences between these studies, but it should be noted that they all recorded MEPs at a single intensity, whereas the present results represent MEP recruitment across a range of intensities. The early significant difference between the 2 hemispheres resolved by 3 months in the present study, but a power analysis suggests that a difference between gradients in the two hemispheres may have been detected at 6 months with a larger patient group. The lack of difference observed here beyond 1 month may have been largely due to a reduction in excitability of the UH.

The relationship between clinical performance and RC gradient was log-linear, with test scores only dropping away when excitability became more severely impaired; this was also the case for motor thresholds. This suggests that function can be well maintained despite significant damage to the corticospinal tract, an idea that receives support from a case series of cerebral peduncle lesions in which 80% of the tract could be destroyed before finger movements were impaired (Jane et al 1968). Recent work used fractional anisotropy to quantify structural disruption of the subcortical white matter tracts at the level of the internal capsules after stroke. In patients with poor motor cortex excitability to TMS motor function dropped off rapidly with increasing corticospinal disruption

(Stinear et al 2007). This reinforces the idea that once a certain level of corticospinal disruption has been reached no amount of upstream reorganisation will be able to generate a useful motor output.

Correlations of MEP amplitudes with clinical scores have been described separately at various time points after stroke. These correlations have been significant at 1 month (Traversa et al 2000), of trend significance at approximately 2 months (Cicinelli et al 1997) and not significant for hand dexterity in the chronic period (Thickbroom et al 2002). While this suggests that the relationship may become less strong with time, it is arguably more suggestive to observe this pattern within a single patient group, as in the present results. It is important to consider what might drive the change in this relationship. In the acute period, 4 patients had gradients at or near zero and these were the most impaired patients. Despite a similar range of relative impairments at 6 months, all patients except one now had easily measurable gradients. Conversely, the patient with the second best recovery at 6 months had a small gradient at this stage. So although this measure reflects function well in the first 3 weeks, the degree of subsequent increase appears not to reflect clinical improvement to such an extent. This is consistent with the idea that continuing behavioural improvement from 3 months relies less on recovery of excitability in the original corticospinal projection than on reorganisation in alternative cortical networks. This would also be in keeping with the finding that the relationship between infarct volume and clinical deficit becomes less strong over this same time period.

Early corticospinal hyperexcitability of the UH has been previously reported, both with the target muscle at rest (Delvaux et al 2003) and active (Cicinelli et al 1997; Traversa et al 1998). In fact, in the present study RC gradients in the UH were never greater than in our control group. The difference may relate to the fact that Delvaux measured MEPs using maximum stimulator output, which may activate pyramidal cells directly rather than trans-synaptically, in which case the MEPs recorded may depend more on spinal excitability (increased following stroke) than cortical excitability. The UH hyperexcitability reported in that study may thus represent a different phenomenon to that observed here. Another possibility is that early UH hyperexcitability may have been more pronounced if MEPs had been elicited from active hand muscles. Reducing UH gradients with time could alternatively be explained by progressive pathological hypoexcitability, for example as a result of diaschisis. However, the absence of clinical correlations at any stage argues against either of these explanations.

3.4.5 Intracortical Excitability

No consistent effects of time were seen in SICI, ICF or LICI in either hemisphere. Mean values calculated for each patient were raised in the affected hemisphere, compared to the healthy group, for SICI and LICI: the larger conditioned MEPs imply that these 2 forms of inhibition were weaker than normal in the affected hemisphere. Correlations with clinical scores were weak during the acute period (except for LICI in the affected hemisphere), strong at 3 months, then weak again at 6 months.

Affected Hemisphere

These measures (SICI, ICF, LICI) all employ a conditioning-test design in which a conditioning stimulus activates inhibitory or excitatory influences on a later test stimulus. The test stimulus needs to evoke a consistent MEP, which in the AH may require a higher intensity than normal and may yield a smaller unconditioned MEP. It is reassuring to note that for the assessment of paired pulse measures the amplitudes of unconditioned MEPs in the affected hemisphere did not differ significantly from those in the unaffected hemisphere at any time point. Moreover, at the 3 month time point (when strong clinical correlations were observed with intracortical measures) unconditioned MEP amplitudes did not co-vary with paired pulse measures in the affected hemisphere. The choice of conditioning stimulus intensity (80% of aMT here) may also cause difficulties. This latter is usually expressed relative to motor threshold, but after stroke the relationship between the corticospinal threshold and the intracortical system being tested may be altered. A thorough study would therefore require testing all these parameters with a range of conditioning intensities (eg Butefisch et al 2003) although this would prolong patient testing considerably.

Our finding of weak SICI in the AH is in agreement with four other studies in which SICI was measured within the first month after stroke (Liepert et al 2000a; Manganotti et al 2002; Nardone et al 2002; Cicinelli et al 2003). One study has reported the converse finding (Wittenberg et al 2007) and the reason for this discrepancy is unclear. There is little information regarding SICI in the chronic stage - we found here that it was fairly stable with no overall effect of time. Normal ICF in the AH has been reported in the first 2 weeks (Liepert et al 2000a) and in the chronic period (Butefisch et al 2003), in

agreement with the present results. There are to our knowledge no prior studies of LICI following stroke. We found that LICI in the AH did not change with time and was significantly weaker than normal. Although LICI shares a dependence on GABA_B receptors with the cortical silent period (Siebner et al 1998; Werhahn et al 1999; Chen 2004), which may be prolonged after stroke (Braune & Fritz 1995; Catano et al 1997a; Liepert et al 2005), recent work suggests that these 2 forms of inhibition may in fact reflect activity in differing neural populations (Inghilleri et al 1996; McDonnell et al 2006). Furthermore LICI, unlike the silent period, does not depend on the ability of the cortex to sustain volitional input, which may be altered after stroke. The weak SICI and LICI observed here thus suggest increased net intracortical excitability.

Unaffected Hemisphere

The stimulus intensity considerations relating to altered motor thresholds do not apply in the UH, where thresholds were normal. No group abnormalities were observed here in measures of intracortical excitability in the UH. However, the presence at 3 months of significant negative clinical correlations with excitability in all 3 measures strongly suggests hyperexcitability in more impaired patients.

We did not observe an overall deficiency of SICI in the UH in the present study. Such a deficiency has been described in patients with cortical but not subcortical stroke (Liepert et al 2000a, 2000b, 2005), although this distinction was not observed in some studies (Manganotti et al 2002; Cicinelli et al 2003). This may be explained by the clinical heterogeneity of the patient group in the present study, or alternatively it is possible that a

difference would have been observed if a range of conditioning stimulus intensities had been used (Butefisch et al 2003). Our finding of normal ICF in the UH is in agreement with a previous study performed 2 weeks after stroke (Liepert et al 2000b), but again the clinical correlation at 3 months implies that it is increased in some patients at this stage. LICI is previously undocumented, and although the group values were normal the same observation applies regarding the situation at 3 months.

3.4.6 Clinical correlations

In marked contrast to corticospinal excitability, intracortical excitability parameters did not correlate well with clinical performance in the acute period (except for LICI in the AH, see below) but showed marked correlations at 3 months. A stratified analysis (not shown) suggests that this change may arise because intracortical excitability was initially raised regardless of eventual outcome, normalising only in patients who recovered well (while persisting or worsening in those who did not). This is in agreement with the study of Manganotti et al (2002), who studied SICI in the unaffected hemisphere at 2 time points within the first month and found that disinhibition resolved in patients with good recovery. Such a relationship between poor clinical status and increased intracortical excitability was not observed when studied previously at a single time point within the first month (Butefisch et al 2003) or in a group of patients at a wide range of time points after stroke (Shimizu et al 2002). However, the present results suggest that the relationship between intracortical excitability and motor function is dynamic, depending crucially on the time since stroke onset. We argue below that this sequence reflects

behavioural improvements shifting from reliance on restored corticospinal function to greater supporting input from intact (and maybe remote) cortical regions.

LICI in the AH differed from the other intracortical measures, correlating well both in the acute period and at 3 months. In the acute period it was also a good predictor of clinical scores at 3 months (not shown). It has been suggested (Orth & Rothwell 2004) that LICI may depend on activity in recurrent axon collaterals from the corticospinal discharge associated with the first (conditioning) stimulus. If LICI thus depends on the integrity of both corticospinal and intracortical populations then its clinical correlations may be expected to reflect both aspects of this mechanism.

3.4.7 Relating physiological changes to clinical recovery

A major finding of the present study is that the corticospinal excitability measures correlated closely with clinical function in the acute period following stroke, and more weakly at 3 and 6 months. Conversely, intracortical excitability measures correlated well at 3 months but not in the acute period (except for LICI in the AH); these correlations were no longer present at 6 months. Indeed, UH intracortical excitability parameters correlated strongly at 3 months despite normal corticospinal excitability (and no corticospinal-clinical correlations) in that hemisphere.

We interpret these findings as suggesting that in the acute period the patient is reliant on whatever remains of the pre-stroke motor output system. Disinhibition is present, releasing connections to adjacent or distant neural populations for use (Jacobs &

Donoghue 1991), but this has not yet been organised into a useful alternative system.

With time and motor practice (eg during physiotherapy), synaptic strengthening may take place in these newly available networks so that as effective a motor output as possible may be generated. Thus by 3 months we suggest that such networks are not only available but organised, and therefore useful. Continued disinhibition is necessary to maintain access to these areas at this stage, so that measures of intracortical excitability correlate with clinical performance.

At 6 months, however, these correlations are greatly reduced. This may reflect decreased reliance on net intracortical disinhibition as training-induced synaptic strengthening becomes better established. It has also been previously suggested that early network reorganisation may with time give rise to permanent structural changes, recently described 5 months after ischaemic infarction in monkeys (Dancause et al 2005). It is unclear whether such structural changes may contribute to the gradual ‘hard-wiring’ of alternative networks. This proposed model of how the generation of a motor output changes after stroke is illustrated in Figure 3.11.

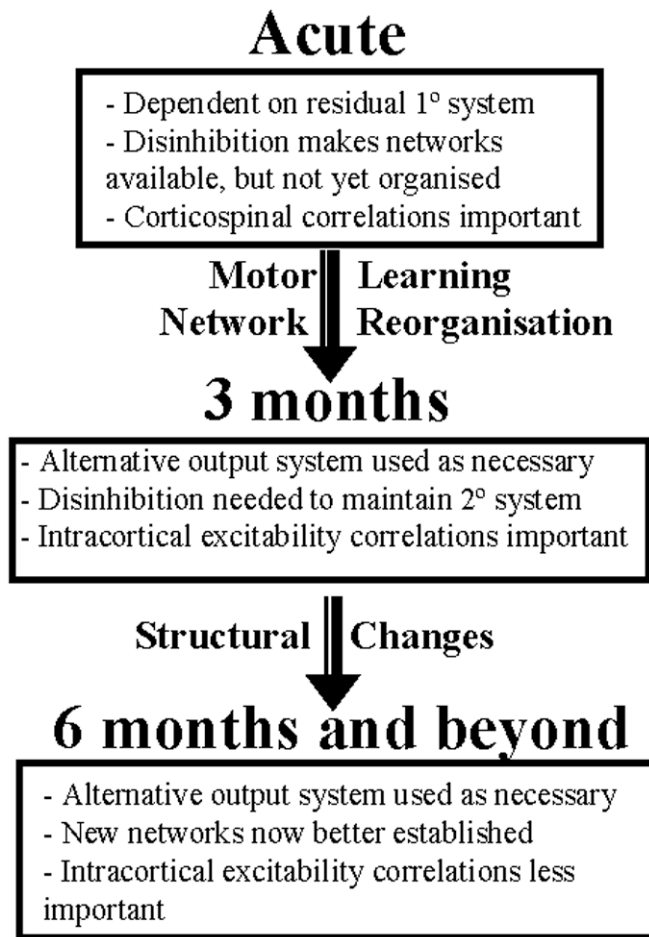


Figure 3.11. Model of physiological changes during stroke recovery

A proposed model is shown for the relationship of physiological changes to recovery from the acute to the chronic stage, compatible with the present results.

It is theoretically possible that the intracortical excitability correlations that we observed at 3 months may simply reflect more severe dysfunction of the wider motor system in more impaired patients, rather than representing an adaptive response as we have argued. It is difficult to separate these possible explanations on the basis of the current data, and to do this would require further work (for example testing the change in such parameters

in response to a treatment intervention). However, it should be noted that between 3 and 6 months we observed a dissociation between clinical function (which did not significantly change) and the intracortical excitability correlations (which were completely abolished). This would be difficult to explain if such correlations were an epiphenomenon of dysfunction, and would be more in keeping with the model we propose.

Post-stroke reorganisation ‘out-sources’ the motor output to a number of non-primary (including contralesional) motor areas. These newly-recruited areas must still maintain some access to the spinal cord, however indirect, in order to assist in generating a motor output. This could be achieved by subcortical projections from these areas or alternatively via the remains of the original motor cortical projection. The second of these possibilities is perhaps the most likely, especially given the persistence of the corticospinal clinical correlations into the chronic period. The role of the contralesional primary motor cortex in this context is unclear: TMS here does not prolong reaction times and exerts abnormally strong transcallosal inhibition during movement preparation (Werhahn et al 2003; Murase et al 2004). Functional roles for the dorsal premotor cortices (PMd) however are well documented, with ipsilesional PMd recruited in less affected patients and contralesional PMd in more affected patients (Johansen-Berg et al 2002; Fridman et al 2004). Furthermore, premotor regions take on M1-like properties in patients with greater corticospinal system damage (Ward et al 2007). This suggests a stepwise recruitment of additional motor areas in the face of damage to the original output system, illustrated in Figure 3.12.

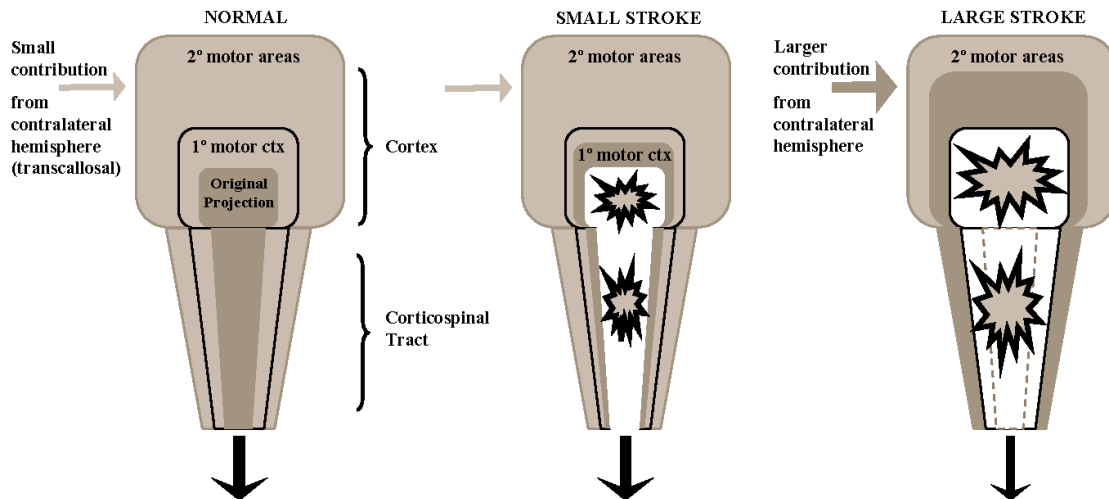


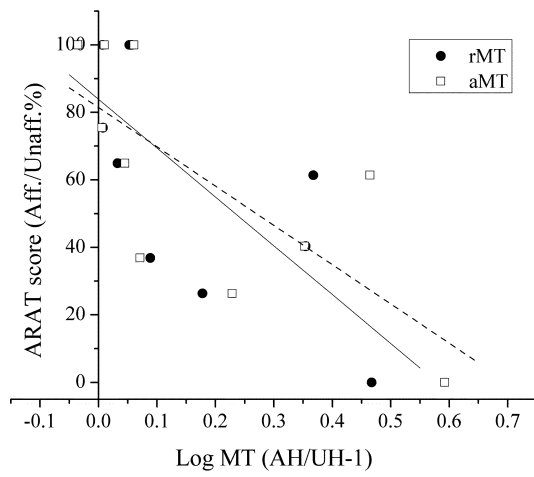
Figure 3.12. Out-sourcing of motor cortical function after stroke

A scheme is shown depicting the possible roles in hand movement of primary and secondary motor areas of one hemisphere. The stroke scenarios are represented in the reorganised (chronic) state. Darker shading denotes greater involvement in movement of the affected hand. A stepwise recruitment of motor areas is depicted with increasing disruption of the corticospinal tract. Minor disruption ('small stroke') results in the recruitment of peri-lesional primary motor cortex, while more extensive disruption ('large stroke') requires the use of secondary motor areas and even transcallosal inputs from the intact hemisphere. We propose that during the subacute stage after stroke a smaller or larger degree of intracortical disinhibition is necessary to maintain access to these additional networks, depending on the extent of disruption of the original corticospinal projection.

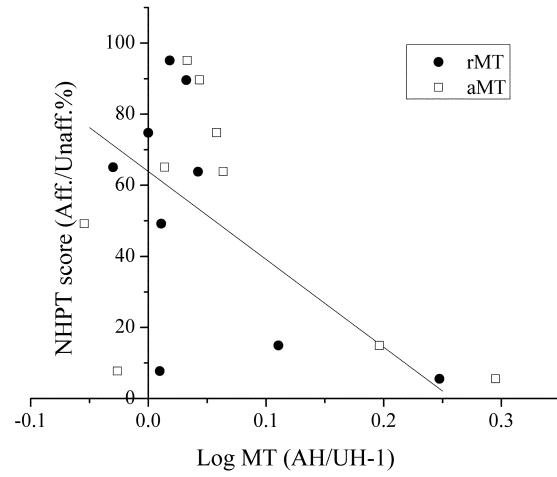
The present results demonstrate that bilateral intracortical disinhibition similarly relates to motor impairment. We propose therefore that at 3 months ongoing disinhibition is facilitating the continued use of these additional networks, and that this disinhibition subsequently becomes less crucial as alternative networks become better established.

Clarification of this model will require larger correlation analyses but is likely to be worthwhile – as the number of novel interventions aiming to enhance recovery grows, it becomes increasingly important to define the physiological framework that underpins motor recovery after stroke.

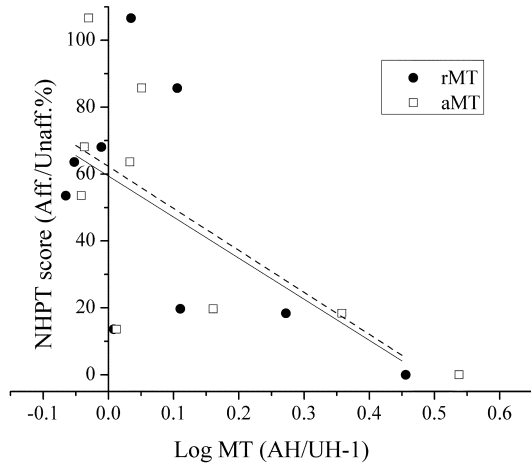
A - Acute period



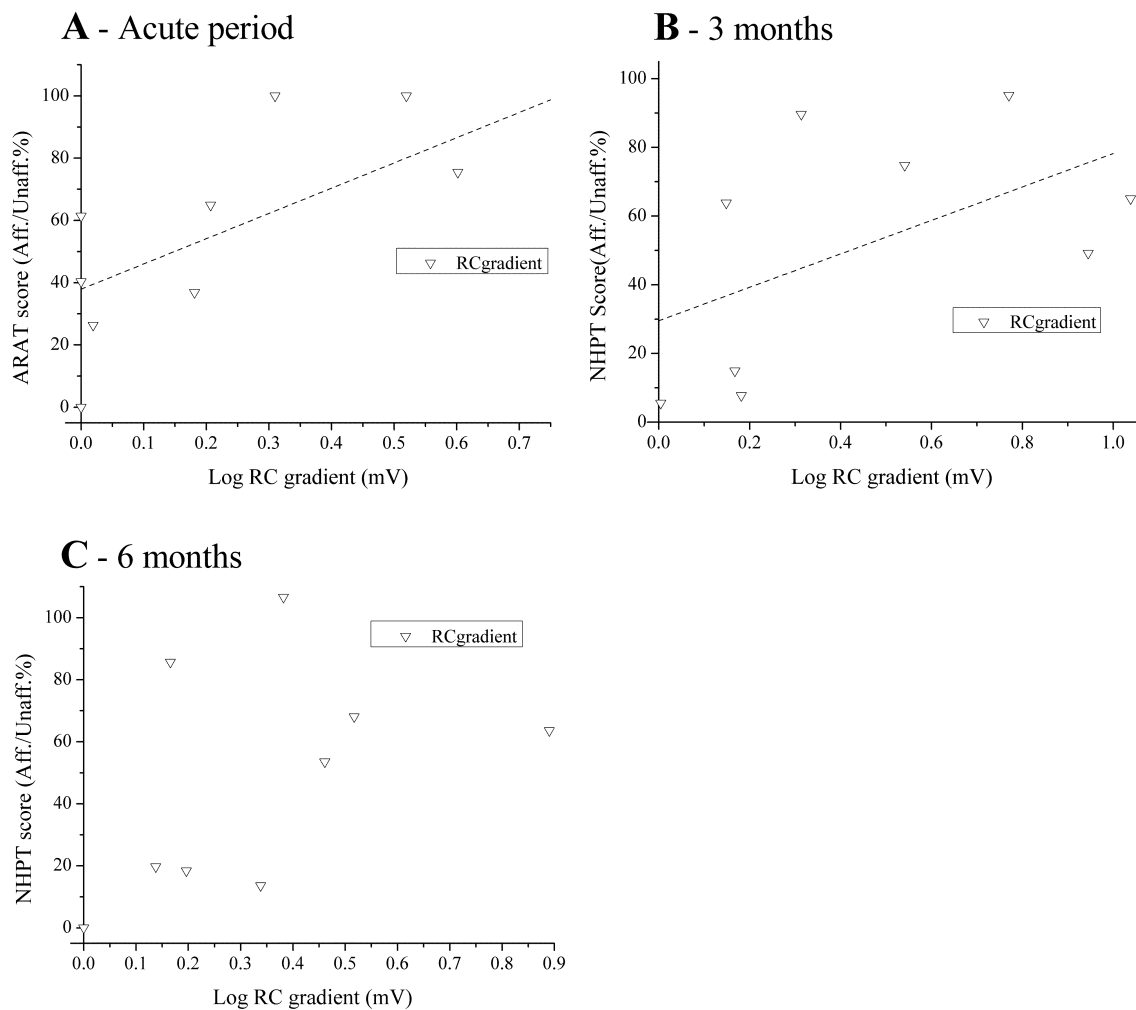
B - 3 months



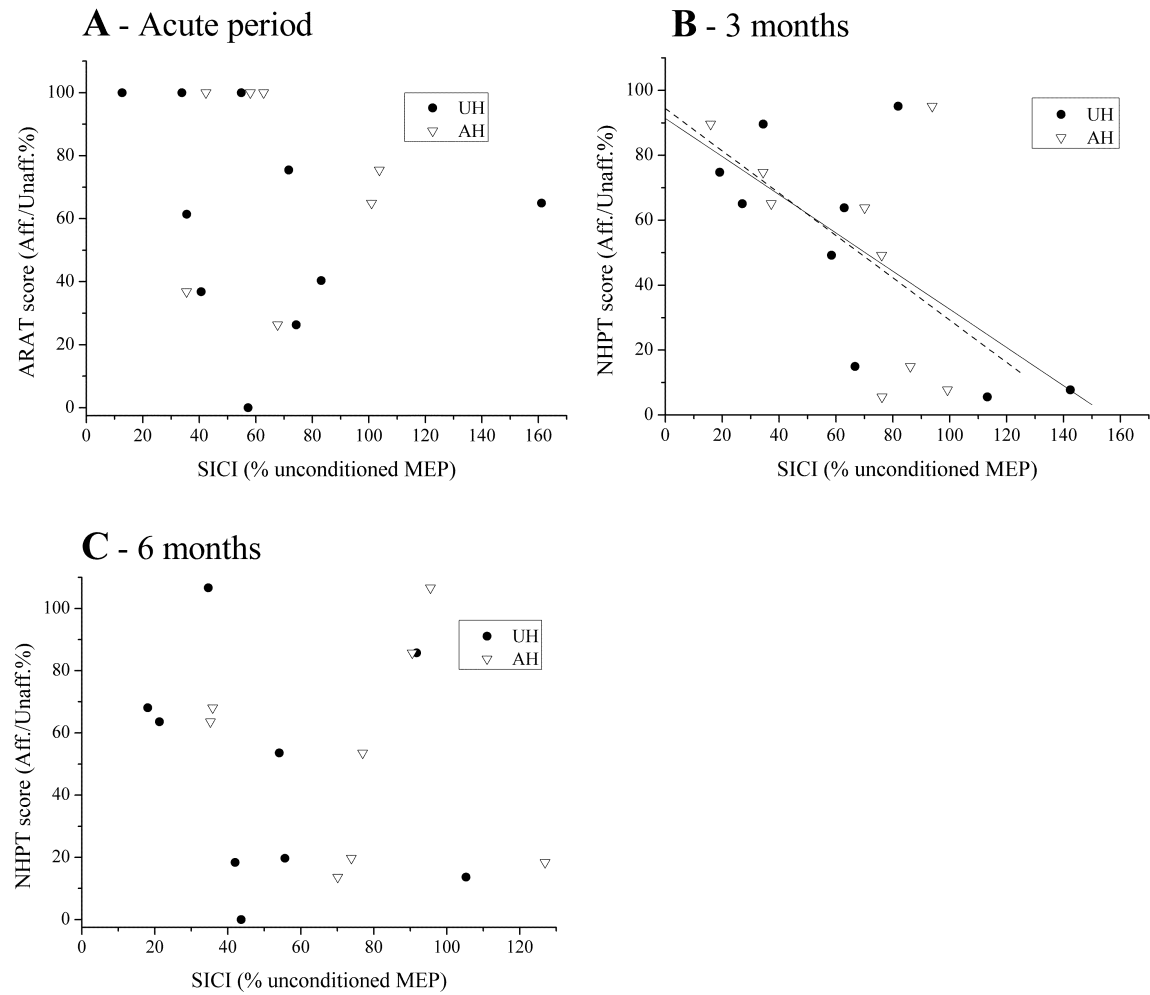
C - 6 months



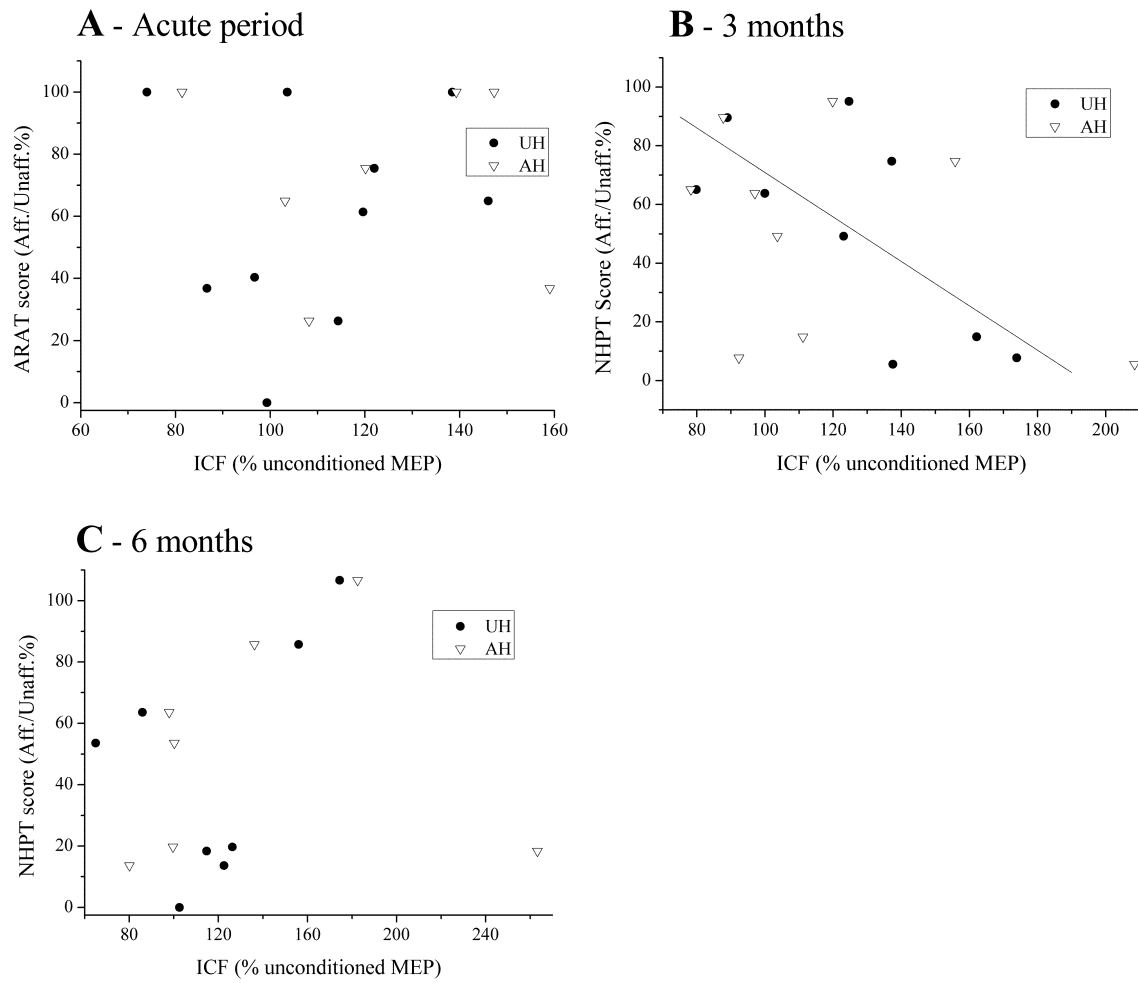
Supplementary Figure 3.1. Motor threshold clinical correlation plots



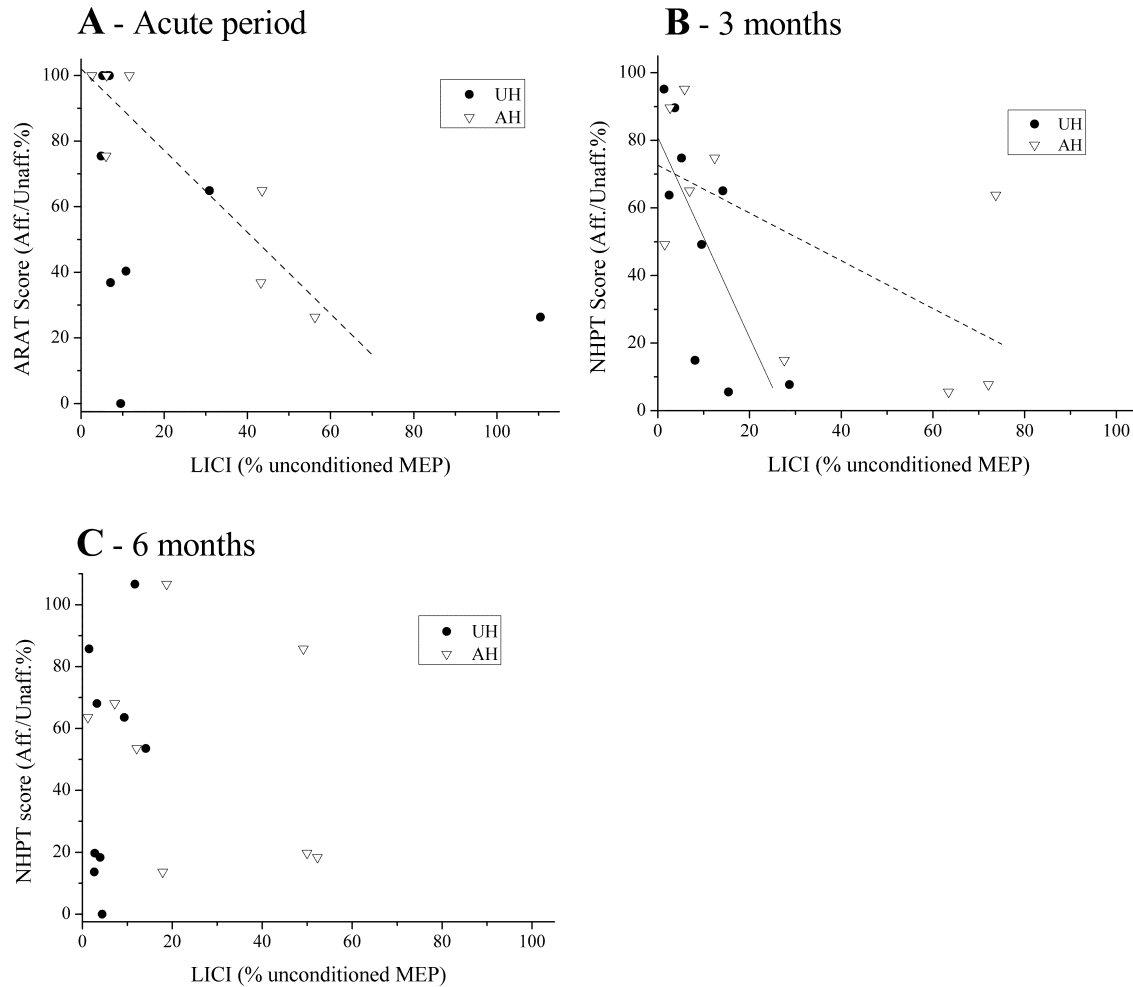
Supplementary Figure 3.2. MEP recruitment curve clinical correlation plots



**Supplementary Figure 3.3. Short Interval Intracortical Inhibition
(SICI) clinical correlation plots**



Supplementary Figure 3.4. Intracortical Facilitation (ICF) clinical correlation plots



Supplementary Figure 3.5. Long Interval Intracortical Inhibition

(LICI) clinical correlation plots

Supplementary Figures 3.1 – 3.5. Complete correlations plots with upper limb scores

The relationships between physiological parameters and clinical scores are shown for the acute period (A), 3 months (B) and 6 months (C).

Within each plot every point represents a single patient. Correlations in the acute period are with the Action Research Arm Test (ARAT) score, while those at 3 and 6 months are with the Nine Hole Peg Test (NHPT) score. The presence of a line of best fit indicates a correlation which is either significant ($P < 0.05$) or a trend ($P < 0.10$) – the complete set of coefficients is given in Table 3.3.

Chapter 4

The role of contralesional dorsal premotor cortex after stroke as studied with concurrent TMS- fMRI

Work described in this chapter was published in Journal of Neuroscience:

Bestmann S, Swayne O, Blankenburg F, Ruff CC, Teo J, Weiskopf N, Driver J, Rothwell JC, Ward NS (2010) The role of contralesional dorsal premotor cortex after stroke as studied with concurrent TMS-fMRI. J Neurosci 30(36):11926-37.

4.1 Introduction

Cortical regions in the intact hemisphere are thought to be important in supporting motor function of the paretic hand after stroke (Seitz et al 1998; Ward et al 2004; Gerloff et al 2006; Lotze et al 2006; Cramer 2008; Schaechter & Perdue 2008; Schaechter et al 2008). Contralateral dorsal premotor cortex (cPMd) is more active during movement of the affected hand after stroke compared with in healthy controls (Chollet et al 1991; Weiller et al 1992), particularly for more impaired patients (Ward et al 2003a) with greater corticospinal tract disruption (Ward et al 2006). Two further lines of evidence suggest that cPMd supports recovered motor function in these patients. First, using transcranial magnetic stimulation (TMS) to disrupt cPMd activity during recovered hand movement can worsen performance in a way not seen in healthy controls (Lotze et al 2006), particularly in patients with greater impairment (Johansen-Berg et al 2002). Second, activity in cPMd when controlling force production with the affected hand is greater in the presence of more extensive corticospinal tract disruption (Ward et al 2007).

However, the mechanisms by which cPMd can exert a functionally relevant causal influence on motor output following stroke remain unresolved. It seems unlikely that cPMd can support hand function via direct projections to spinal cord motoneurons (Boudrias et al 2010). An alternative hypothesis is that cPMd might influence other cortical areas in the surviving motor network to support residual motor output (Cramer 2008) and higher-order processes required for motor function (Gerloff et al 2006). Paired-coil TMS studies have demonstrated a direct inhibitory interhemispheric influence of PMd on the output of primary motor cortex (M1) in the opposite hemisphere for healthy

humans at rest (Mochizuki et al 2004; Baumer et al 2006; Koch et al 2006; O'Shea et al 2007b). During an active motor task, this inhibitory influence can change (Koch et al 2006; O'Shea et al 2007a; Bestmann et al 2008b). We assessed the influence of cPMd on surviving cortical motor regions in stroke patients with different levels of impairment to test how cPMd might support motor function in the face of partial corticospinal tract disruption via an influence on other brain areas. Furthermore, we asked whether such influences are state dependent, i.e. change from rest to active movement of the paretic hand.

First, we tested the direct interhemispheric influence of cPMd on ipsilesional M1 in subcortical stroke patients at rest using paired-coil, paired-pulse TMS (Mochizuki et al 2004; Baumer et al 2006; Koch et al 2006). In a separate experiment with the same patients, we used concurrent TMS-fMRI to deliver TMS pulses over cPMd while measuring its causal influence on brain activity during hand grip or rest in other potentially interconnected brain areas. We sought to explain variability in the state-dependent (i.e. hand grip or rest) influence of cPMd as a function of clinical and neurophysiological impairment. We thus tested which parts of the surviving motor network were causally influenced by cPMd during affected hand grip, and how this influence might vary with the level of residual motor function or in relation to the separately assessed paired-coil neurophysiological measure.

In this chapter all TMS and scanning experiments were performed both by the author and by Dr Sven Bestmann. The TMS data was analysed by the author, while statistical parametric analysis of the MRI data was performed by Dr Bestmann.

4.2 Methods

4.2.1 Participants

Patients were recruited from the National Hospital for Neurology and Neurosurgery, Queen Square, London. All were pre-morbidly right handed and had experienced a first-time ischemic stroke resulting in weakness of (at least) wrist and finger extensors and hand interossei lasting a minimum of 48 hours (mean time after stroke 28 months, range 4 -104). Exclusion criteria were: 1) extension of the lesion into cortical motor regions; 2) carotid artery stenosis $\geq 70\%$ as assessed by carotid doppler studies and/or magnetic resonance angiography; 3) previous seizures or other neurological or psychiatric diseases; 4) inability to perform the grip task used during the fMRI part of our study (see below); 5) deficits of language comprehension; and 6) time after lesion < 4 months.

Patients were not receiving active physical therapy, but had received post-stroke therapy appropriate to their individual clinical needs. All patients (age 57.4 ± 11.6 years) and had normal (or corrected-to-normal) vision. Patients were naïve to the purpose of the experiment. Twelve patients (2 female) were included in the final experiment. Patient characteristics are listed in Table 4.1. Structural brain images with markers of the lesion are given in Figure 4.1.

Table 4.1

Patient	Sex	Age (Years)	Site of Lesion	Time Since Stroke (months)	Past medical history	Medication
1	M	52	L stratiocapsular	22	Hypertension, AF, DM,	Warfarin, Insulin, Ramipril, Bendrofluazide
2	M	52	R stratiocapsular	104	Hypothyroidism	Aspirin, Pravastatin, Thyroxine
3	F	55	R stratiocapsular	6	Hypertension, COPD, Mild depression	Aspirin, Ezetimide, Omeprazole, Salbutamol
4	M	46	R stratiocapsular	31	Nil	Aspirin
5	M	59	L pons	13	Hypertension, DM, Gout	Aspirin, Diltiazem, Gliclazide, Metformin, Simvastatin, Omeprazole
6	F	84	R stratiocapsular	26	IHD, Hyperthyroid, AF	Aspirin, Digoxin, Atenolol, Thyroxine, Ramipril
7	M	55	R internal capsule	17	Hypertension, DM, Renal Impairment	Aspirin, Dipyridamole, Insulin, Frusemide, Diltiazem, Ramipril, Atorvastatin
8	M	75	R internal capsule	13	Hypertension	Aspirin, Simvastatin
9	M	51	R corona radiata	4	Diabetes	Warfarin, Gliclazide, Simvastatin
10	M	58	R stratiocapsular	9	AF	Warfarin, Flecainide, Bisoprolol, Atorvastatin
11	M	43	R thalamocapsular	11	Hypertension	Bisoprolol, Lisinopril, Amlodipine, Bendrofluazide
12	M	59	R thalamus/insula	84	Hypertension, AF	Warfarin, Simvastatin, Propranolol, Flecainamide, Perindopril, Amlodipine

Table 4.1. Patient details

M, Male; F, Female; L, Left; R, Right; AF, Arterial Fibrillation; COPD, Chronic Obstructive Pulmonary Disease; DM, Diabetes; IHD, Ischaemic Heart Disease. NHPT, Nine Hole Peg Test; * affected limb scores as a percentage of unaffected limb scores.

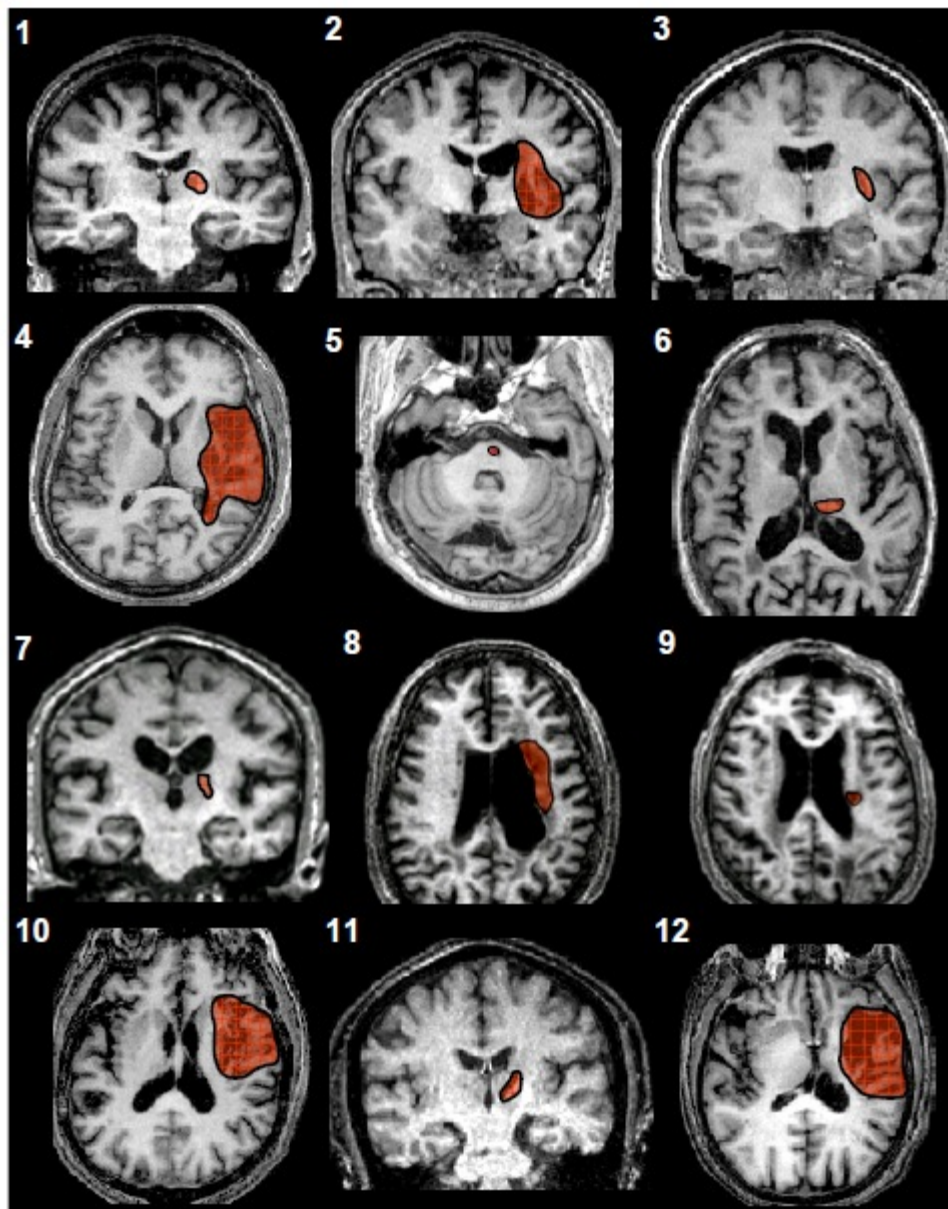


Figure 4.1 Structural MRI images showing lesions

Axial and coronal T1-weighted anatomical MRI scans at the approximate level of maximum infarct volume (indicated by red region) for each patient. For two cases, images are flipped so that all lesions appear on the right side.

4.2.2 Clinical evaluation

Patients were evaluated at the time of scanning using the nine-hole peg test (NHPT, Heller et al 1987), Motricity index in the upper limb (MI-UL), and action research arm test (ARAT; see Table 4.2).

Table 4.2. Clinical scores

Patient	NHPT	ARAT	MI-UL
1	92.4	55	100
2	60.6	57	100
3	68.1	57	100
4	43.0	36	89
5	85.7	57	100
6	68.3	55	81
7	69.7	51	100
8	70.5	55	100
9	38.6	56	77
10	0	15	56
11	76.3	57	100
12	5.0	25	77
Mean \pm Std	56.5 \pm 29.5	48 \pm 14.5	90 \pm 14.4

See Chapter 2 for details of NHPT and ARAT. The NHPT score is expressed as the percentage of pegs per second for the affected hand with respect to the unaffected hand. The MI reflects a clinical assessment of power in 3 muscle groups of the upper limb. To obtain an overall index of residual motor function that is less affected by floor or ceiling effects that may arise when only taking one score into account, we derived a combined clinical score, using the first principal component of a principal component analysis (PCA) of the clinical assessment scores as described in previous work (Ward 2003a, 2003b). In the resulting combined score, a positive score denotes better residual function while a negative score denotes poorer residual function.

4.2.3 Interhemispheric cPMd-iM1 paired-coil TMS

In addition to the clinical assessment, we sought to obtain a physiological measure of the integrity or otherwise of normal interhemispheric PMd-M1 influences, using paired-coil TMS (Koch et al 2006; Civardi et al 2001; Mars et al 2009; O'Shea et al 2007b). TMS was performed within one day of the scanning session (see below) using two MAGSTIM 200 stimulators (The Magstim Company, Dyfed, UK). We measured: (i) resting motor threshold (rMT) and active motor threshold (aMT) for each hemisphere; (ii) interhemispheric influences from cPMd to ipsilesional M1 (cPMd-iM1) using a previously described protocol (Mochizuki et al 2004). This physiological measure was acquired at rest only, because during voluntary action I-waves in motor cortex are recruited differently than at rest (Amassian & Stewart 2003). This makes it difficult to ascertain whether any stroke-related changes in motor-evoked potentials (MEPs) in an active setting are caused by a change in I-wave recruitment or the stimulation of different

intracortical pathways. Here, we were primarily interested in obtaining a physiological measure (separate from fMRI data) of cPMd-iM1 influences after stroke, and using this to explain variability in residual motor function, or indeed differences found in brain responses during concurrent TMS-fMRI across our patient group.

For motor threshold measurement, the handle of a 70 mm diameter figure-of-8 coil was held pointing postero-laterally over the M1 hand representation, defined as the position at which stimulation produced consistent MEPs in the target FDI. See Chapter 2 for definitions of resting and active MTs. For the paired-coil protocol probing the interhemispheric influence of contralesional PMd on ipsilesional M1, a small TMS coil (figure-of-8 shape, 50 mm diameter) was placed over PMd by locating it 2 cm anterior and 1 cm medial to the motor-hotspot (see also (Johansen-Berg et al 2002; Bestmann et al 2005; O'Shea et al 2007b; Schluter et al 1998; Schluter et al 1999; Bestmann et al 2008b for similar PMd coil locations), with the handle pointing laterally for a medially-directed induced current (Mochizuki et al 2004; Koch et al 2006). A second coil (figure-of-8 shape, 70 mm wing diameter) was placed over the contralateral M1 hand representation, as described for motor threshold measurement above. A conditioning stimulus (CS) was applied over cPMd, 8 ms before a test stimulus (TS) over iM1, with the CS initially set at 100% of rMT for the unaffected hemisphere. The TS intensity over iM1 was then adjusted in each patient to evoke an unconditioned motor evoked potential (MEP) of approximately 1 mV amplitude. If it was not possible to obtain an MEP of this amplitude then the lowest intensity still producing a stable MEP was used. Conditioned trials (CS-TS) were randomly interleaved with unconditioned trials (TS alone) during the paired-

coil protocol. A minimum of 15 trials of each condition was recorded. All MEPs in response to iM1 pulses were recorded by surface EMG using a belly-to-tendon montage from the first dorsal interosseous muscle (FDI). The raw signal was amplified and filtered with a band-pass filter of 30 Hz to 1 kHz (Digitimer Ltd). Signals were digitized at 2 kHz (CED Power1401, Cambridge Electronic Design, Cambridge, UK) and stored on a laboratory computer for offline analysis.

4.2.4 Concurrent TMS-fMRI

Concurrent TMS-fMRI can provide insights about causal interactions among brain regions and help to establish causal brain-behaviour relations for the human brain, not only at the local site targeted with TMS but also for remote interconnected brain regions (Sack et al 2007; O'Shea et al 2007a; Bestmann et al 2008a; Driver et al 2009). Here we used this approach in a 2 x 2 factorial event-related design in which each trial consisted of an instruction to perform a single affected hand grip or to maintain rest, and a concurrent high- or low-intensity TMS over cPMd (at mean 79% or 44% of maximal stimulator output intensities, respectively; see below). TMS at these intensities was applied during hand grip or rest with equal probability.

4.2.4.1 Experimental paradigm

During scanning, a visual cue on each trial indicated that participants should either perform a single brief isometric hand grip with their affected hand, or maintain rest. Hand grips were performed using an MR-compatible manipulandum consisting of two force transducers (Honeywell FSG15N1A; Honeywell, NJ, USA) situated between two

moulded plastic bars (width 6 cm). Compression of the two bars by isometric hand-grip resulted in the generation of a differential voltage signal, linearly proportional to force exerted, which was fed into a signal conditioner (CED 1902; Cambridge Electronic Design, Cambridge, UK). This signal was digitized (CED 1401; Cambridge Electronic Design, Cambridge, UK) and fed into a computer running Cogent 2000 (see <http://www.vislab.ucl.ac.uk/cogent.php>). The dynamic change in recorded signal was projected in real time onto a screen to give visual feedback to each participant, as a column for which the height varied linearly with change in voltage and hence with force (see Figure 4.2 for examples of the visual display). Prior to scanning, but while lying in the scanner, subjects were asked to grip the manipulandum with maximum force to generate their maximum voluntary contraction (MVC).

Figure 4.2

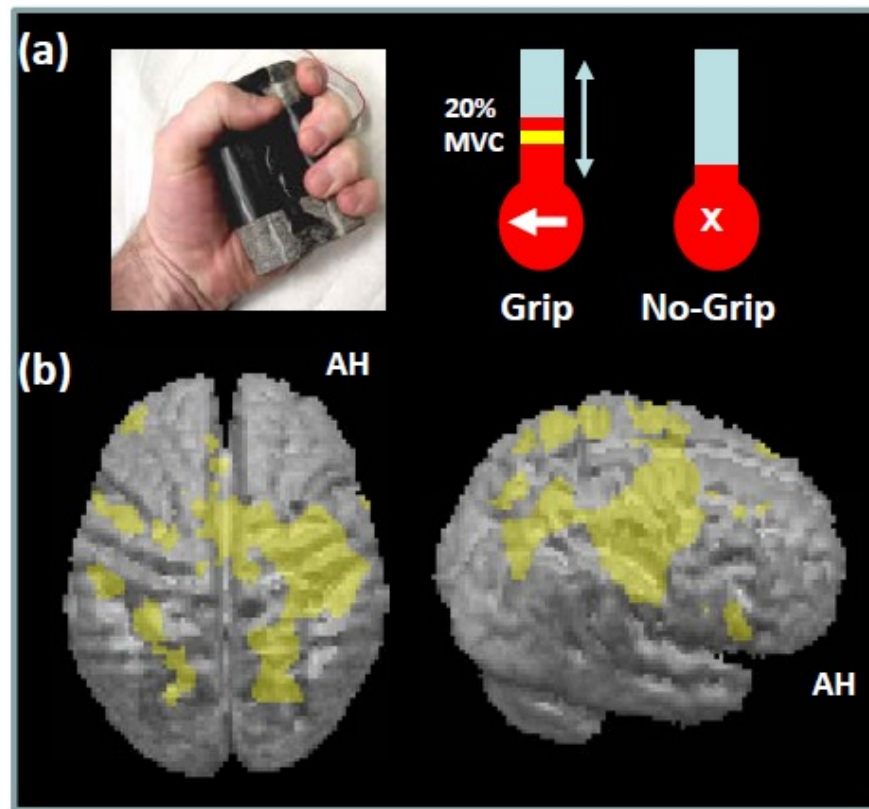


Figure 4.2.

Experimental setup and main effects of the grip task

(a) Photograph of grip-force manipulandum shown at left, together with two example schematics of screen displays during scanning, for a *GRIP trial* (as instructed by arrow shown at bottom of thermometer-like visual display), the other for a *NO-GRIP trial* (as instructed by a central cross being presented instead of the arrow). During *GRIP* trials, a yellow target bar indicated the required force level as in the schematic example. The actual force exerted by the weak hand was indicated online by red shading of the thermometer-like display, while the white arrow pointed to the paretic hand to indicate that active grip was required. Participants were instructed to generate a non-ballistic force matching the displayed target bar,

using a gentle pace without major corrective movements (see main text). On all trials, TMS (5 pulses, 11 Hz) was applied unpredictably to contralesional PMd at one of two intensities (110% resting motor threshold or 70% active motor threshold), 900 ms after presentation of the force target level (or cross) visual instruction.

(b) Grip-task-related activity, irrespective of TMS. The results of the group random effects analysis are projected onto the rendered averaged structural scans from all patients. The height threshold was set at $T > 3.5$, uncorrected for multiple comparisons across whole brain, and the extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across the whole brain.

At the start of each trial, the requirement for an active hand grip (if applicable) was indicated visually by an arrow pointing to the side of the affected hand displayed at the bottom of the screen for 3 seconds (see Figure 4.2a). The appearance of this arrow indicated that the subject was to perform a single brief handgrip with the affected hand, to be continued until the column representing the force applied came into contact with a horizontal bar on the screen (indicating the target force of 20% of the affected hand's MVC on the day of scanning), at which point the grip could be released. In an equal number of unpredictable intermingled trials, a cross was presented instead to indicate that subjects should maintain rest. In this case, participants kept their hands relaxed. Participants held one additional manipulandum in their unaffected hand to record any mirror movements, or undesired twitches during TMS caused by a possible spread of excitation from the stimulated cPMd into adjacent cM1. With this setup, we confirmed outside of the main experiments that TMS-induced finger movements could indeed be detected reliably by the force transducers when TMS similar to our main stimulation protocol was applied to contralesional M1 instead (5 pulses at 11 Hz and 110% RMT). No finger twitches were observed during TMS in this case, demonstrating that contralesional M1 was not stimulated by the cPMd-TMS protocol applied during the main experiment.

In addition to the instruction to grip or rest during fMRI, as part of each 'event', TMS was applied to cPMd inside the scanner unpredictably at either 110% of individual rMT (TMS_{high} condition) or 70% of aMT (TMS_{low} condition) on each trial. Resting MT during scanning had been determined visually for the unaffected hand when stimulating over

contralateral M1. In the TMS_{low} condition, stimulation intensity was 56% (44 ± 7 maximum stimulator output, MSO) of the TMS_{high} condition (79 ± 11 MSO). Therefore, TMS_{high} was assumed to exert significantly stronger effects on cortical processing, compared to TMS_{low} , as directly assessed by contrasting the two conditions in our fMRI analysis (see below). Note that these thresholds (and corresponding stimulation intensities for TMS_{high} and TMS_{low}) for the fMRI session differ (see below) in terms of MSO from the motor threshold obtained separately outside the scanner because visually determined thresholds are slightly higher than motor thresholds determined using electromyography, and because the increased impedance of the extended MR-compatible cable running from the stimulator to the coil during the scanning experiment increases the TMS stimulator output required for the same degree of cortical stimulation.

Each TMS train during scanning comprised 5 pulses at 11 Hz, starting 900 ms (10 EPI slice acquisitions) after presentation of the instructional visual cue. Previous work in healthy subjects had shown that, with this interval, TMS-pulse application coincided well with the neural activity related to grip force generation (Bestmann et al 2008b). A single scanning session comprised one hundred trials (twenty each of TMS_{high} -GRIP, or TMS_{low} -GRIP, or TMS_{high} -REST, or TMS_{low} -REST, together with twenty null events). Each patient therefore received a total of 400 TMS pulses during the main experiment, in line with currently available safety recommendations (Rossi et al 2009). The inter-trial intervals varied unpredictably between 11-21 seconds (mean 16.11 seconds). These conservatively long inter-trial intervals were chosen to preclude carry-over effects between TMS trials with the stimulation used (Modugno et al 2001; Gilio et al 2007). Moreover, the TMS

aspects of our design (high or low-intensity) were event-related and had a pseudo-random order, so that any possible carry-over effects should not contribute to our comparisons in any case. Trial order was pseudo-randomised so that each trial type occurred twice within ten consecutive trials.

Patients were trained outside the scanner until comfortable with the grip task, without TMS being applied. Inside the scanner, the TMS coil was positioned over cPMd (see below). The task was briefly practised again, ensuring that patients performed brief but non-ballistic isometric hand grips that reached or approximated the required force level on every active trial with only the contralesional hand. We explicitly instructed patients that speed was not critical and that they should generate a non-ballistic handgrip to approximately match the displayed target bar using a gentle pace without major corrective movements (as also done in Bestmann et al 2008b). These instructions ensured that patients could perform the task without difficulty, and rendered it less likely that TMS would induce any systematic changes in behavioural. This was important for our approach, and a simple non-speeded motor task was also used in our previous study on healthy participants (Bestmann et al 2008b).

A few trials were performed inside the scanner with TMS being applied at 110% rMT while patients were contracting their unaffected hand (i.e. contralateral to TMS) at around 20% MVC. This allowed a further check that indeed no twitches were induced by cPMd TMS (neither ipsilateral nor contralateral to the PMd). No overt muscle responses were

observed in any participant, nor later reported by participants, other than the intended grip with the weak hand when required by the grip task.

4.2.4.2 Data Acquisition

See Methods Chapter (2.5) for details.

4.2.4.3 Concurrent TMS-fMRI

TMS pulses were applied during the dead time between the EPI navigator echoes and the EPI data readout, and separated from RF slice excitation pulses (Bestmann et al 2003). Each slice coincided equally often with TMS pulses to avoid any systematic influences on slice-by-slice variance. The TMS coil was oriented tangential to the scalp, approximately perpendicular to the precentral sulcus. This induced a biphasic current with an initial antero-posterior induced direction relative to the axis of the coil. Following scanning, the PMd site that had been stimulated inside the scanner was reconstructed from T1-weighted structural scans which included five fiducial markers placed at the centre of the two TMS coil wings, their anterior bifurcation, and the left and right side of the coil cable at their posterior bifurcation (see Figure 4.3). The stimulation site was thereby determined as the intersection between the imaged cortex and a line going through the centre of the TMS coil and perpendicular to the plane of the figure-of-eight coil. The reconstructed TMS coil position for each participant confirmed that the stimulation location was clustered within PMd anterior to the precentral gyrus, and dorsal to the intersection of the medial frontal sulcus and precentral sulcus (Amiez et al 2006) (see Figure 4.3).

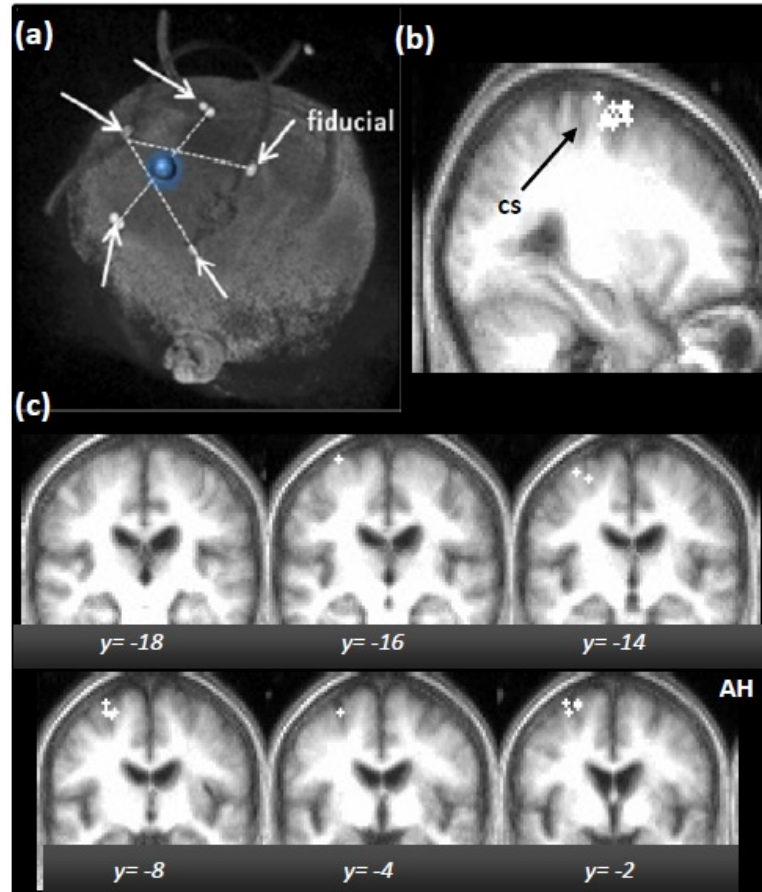


Figure 4.3. Cortical stimulation site

(a) Example of the position of the fiducial markers attached to the MR-compatible TMS coil for one participant, with the centre of the coil indicated in blue. (b, c) Individual stimulation locations. Each white cross indicates the MNI coordinate of the derived stimulation site for a single patient, with a summary shown on a sagittal view of the mean structural image at right, and example coronal slices at left. The group mean stimulation point was (mean \pm std) $x = -29 \pm 4$, $y = -9 \pm 5$, $z = 65 \pm 4$. Coronal and sagittal sections of the mean T1-weighted structural scans (averaged across all patients) are shown. AH: affected hemisphere (shown on right in each coronal example); cs: central sulcus.

4.2.5 Data analyses

4.2.5.1 Behavioural data during fMRI

Movement onset, grip duration, and peak force were measured for each trial. Movement ‘onset’ was defined as the latency between cue onset and the point at which grip force exceeded 20% of baseline value. Note that cortical activity related to grip force production will start several tens or hundreds of milliseconds before this level was reached. Together with the TMS train duration of 360 ms this ensured that cPMd stimulation would overlap with grip-related activity during grip trials (see also Bestmann et al 2008b). Grip duration was determined as the interval between the successive time points at which grip force started to exceed or started to fall below the 20%-of-baseline boundary on each trial. The peak force was determined as the maximum force during this period for each trial. Paired two-tailed *t*-tests were used to compare these parameters between high/low TMS intensity conditions across participants. Our explicit aim was to use TMS during scanning to probe local and remote activity changes directly (O'Shea et al 2008; Bestmann et al 2008a; Paus 2005), rather than to produce behavioral changes that might then complicate interpretation of any fMRI changes also associated with TMS. Consequently, our instructions did not require patients to perform the movements at high speed or accuracy (see also Bestmann et al 2008b for a closely analogous approach in healthy participants), and we therefore did not *a priori* expect any TMS impact on performance in the grip task.

4.2.5.2 Interhemispheric cPMd-iM1 influences as assessed with paired coil TMS

Individual trials were examined off-line and those showing any voluntary EMG activity were discarded. Peak-to-peak MEP amplitudes were measured in the remaining trials using in-house software. The causal influences from cPMd upon iM1 were calculated in each patient as the mean amplitude of conditioned MEPs expressed as a percentage of unconditioned MEPs. The linear partial correlation between the paired-coil cPMd-iM1 measure and the combined clinical score was calculated using a partial correlation procedure, thus controlling for additional variables that may explain the observed cPMd-iM1 influence ($r_{MT_{AH}}$ and test pulse MEP size).

4.2.5.3 fMRI analysis

See Chapter 2 for details of fMRI data pre-processing. To allow a unified statistical model, images from the only two patients with a left-hemispheric lesion were flipped about the sagittal plane to permit statistical comparison across participants, i.e. shifting the ipsilesional hemisphere to the right for all cases (Ward et al 2003a; Ward et al 2006; Ward et al 2007).

Statistical analysis of the fMRI data involved two stages. First, a single subject fixed-effects model was computed for each participant by multiple regression of the voxel-wise time series onto a composite model containing the covariates of interest. Each of the four event-related trial types (TMS_{high} during GRIP, or TMS_{low} during GRIP, or TMS_{high} during REST, or TMS_{low} during REST) were modeled as delta functions, with onsets defined as the first TMS pulse, and were included as separate covariates. To account for any additional variance induced by any slight trial-by-trial and/or inter-subject variation in

grip onset, grip duration, and grip force, these parameters were included as parametric modulations that scaled the delta function representing the onset of each grip trial.

All covariates were convolved with a canonical synthetic hemodynamic response function in a general linear model (Friston et al 1995; Friston et al.1998), together with a single covariate representing the mean (constant) term over scans. The parameter estimates for each covariate resulting from the restricted maximum-likelihood fit of the model to the data were calculated. Statistical parametric maps of the t-statistic resulting from linear contrasts of covariates (Friston et al 1995) were generated and stored as separate images for each subject. For each subject we calculated (i) the effect of GRIP minus REST (irrespective of TMS strength), (ii) the effect of HIGH minus LOW intensity TMS to cPMd (irrespective of whether gripping or not), and (iii) the 2-way interactions between these factors. Second, the group level random effects analysis comprised parameter estimates for each of these contrasts across all subjects. Contrast images from each subject were entered into a one sample t-test, for each contrast of interest. The height threshold for the resulting SPMs was set at $T > 3.5$, and the extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across whole brain.

In order to exploit heterogeneity in our patient group, we tested whether any between-subject variability in these TMS-fMRI effects related to the degree of residual motor function, as indexed by our combined clinical assessment score for each patient. In addition, we were interested in whether the degree of interhemispheric influence from

cPMd to ipsilesional primary motor cortex (as assessed with our separate paired-coil cPMd-iM1 measure) could explain variability in our concurrent TMS-fMRI results. Because cPMd-iM1 influences at rest also turned out to be related to residual motor function in our patients (as measured by a combined clinical score), we asked whether this cPMd-iM1 paired-coil measure might provide any additional explanatory power for the fMRI results, above and beyond the combined clinical score. We therefore performed a linear regression analyses within SPM5, within the contrast images for each subject, with the effects of interest now being regressed upon separate values representing the degree of residual function and (separately) the paired-coil cPMd-iM1 measure for each subject. The latter two measures were both entered into the same second-level model as additional covariates. We first considered the combined clinical score because previous work has shown some relation of motor task-related activity to residual motor function (Ward 2006). We then considered the cPMd-iM1 measure to see if any additional fMRI variance might be explained by this further physiological measure of interhemispheric influence.

To illustrate the relationship for significant peak voxels, we plot the extracted parameter estimates against the combined clinical score, and separately against the paired-coil cPMd-iM1 measure, respectively. But note that this is merely to provide some visualisation of the relationship between the variables tested. The formal analysis to address this was performed via the SPM linear regression analysis and associated statistics.

4.3 Results

4.3.1 Behavioural results during scanning

All patients were able to perform the grip task adequately (Figure 4.4)

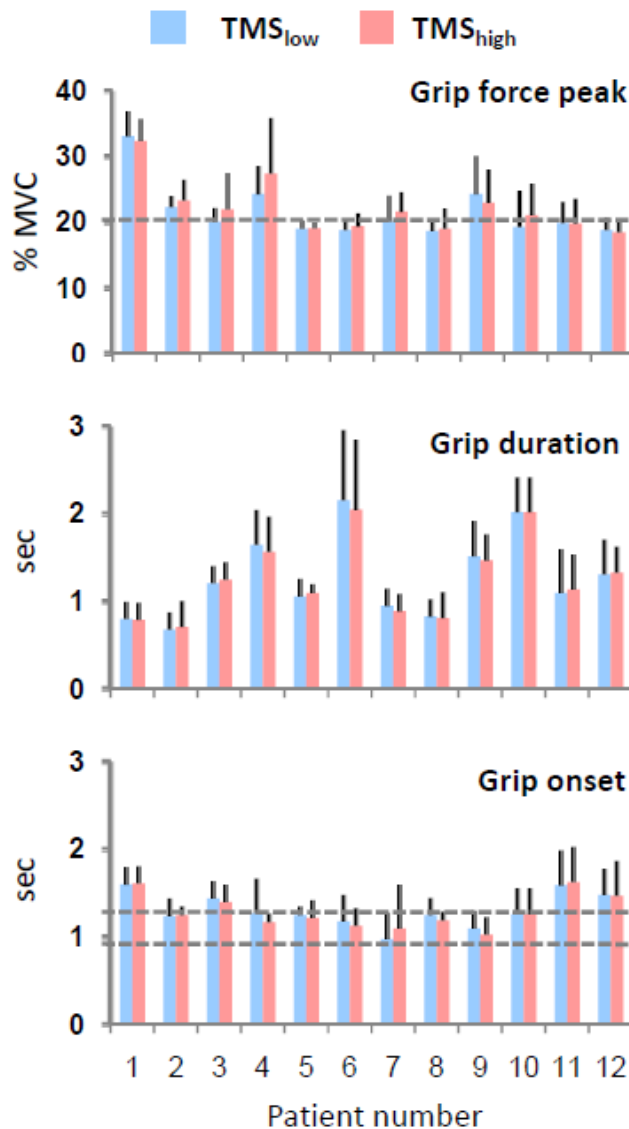


Figure 4.4.

Average grip peak-force, duration and onset time for each patient, shown separately for TMS_{low} (blue bars) or TMS_{high} (pink bars) trials, with adjacent bars for each patient. Data are presented as means \pm SD. For peak force, the dotted line indicates the required force level. For

grip onset, the dotted line represents the period of TMS pulse application. Note that TMS did not significantly affect scanning in any patient. MVC: maximum voluntary contraction.

Each patient's clinical picture was dominated by motor impairment with little or no sensory loss. The Ashworth scale for spasticity was zero in all patients. No patient displayed mirror movements or synergistic flexor movements in more proximal joints, neither when assessed outside the scanner by direct observation, nor during scanning, as confirmed by inspection of the sensitive force recordings from the unaffected hand during movement of the affected hand. The grip onset times (mean \pm std: $1.3\text{s} \pm 0.19$ for TMS_{low} , $1.28\text{s} \pm 0.2$ for TMS_{high}) indicate that TMS overlapped with neural processes associated with active grip generation, as in our recent TMS-fMRI study of healthy subjects (Bestmann et al 2008b). The comparison of task performance between the TMS_{high} and TMS_{low} conditions was not significantly different for any grip parameter (grip onset: $t_{11}=0.92$, n.s.; grip duration $t_{11}=0.90$, n.s.; peak force: $t_{11}=1.82$, n.s.). Thus, high-intensity TMS during scanning did not significantly change motor behaviour compared to low-intensity TMS, consistent with our intention of avoiding any significant behavioural effects of TMS during scanning that might otherwise have complicated interpretation of TMS influences on the fMRI data (see also Bestmann et al 2008b; Ruff et al 2006).

We were specifically interested to examine variability in the causal influence of cPMd on surviving brain regions as a function of (i) residual upper limb impairment and (ii) the resting influence of cPMd on iM1 as assessed separately using paired-coil TMS. The

results from the cPMd-iM1 paired-coil TMS experiment and their use as explanatory variables is incorporated into the results described below.

4.3.2 Interhemispheric paired coil TMS

We measured the influence of cPMd on iM1 using paired-pulse TMS outside the scanner in a separate session. We found that the influence of cPMd on iM1 at rest was inhibitory (with the conditioning cPMd pulse reducing the MEP evoked by the iM1 test pulse) in some patients, similar to healthy controls (Mochizuki et al 2004). But notably, in other patients, the conditioning stimulus to cPMd resulted in less inhibition or even facilitation. This finding was related to the combined clinical score ($r = -0.62$, $p < 0.05$). Thus, in patients with minimal motor impairment, interactions between cPMd and iM1 at rest were predominantly inhibitory, similar to those previously observed in healthy subjects (Mochizuki et al 2004; Koch et al 2006; O'Shea et al 2007b). In contrast, in patients with greater impairment, this influence tended to be reversed, demonstrating that the paired-coil TMS approach can highlight impairment-specific differences in the influence of cPMd on iM1 (Table 4.3; Fig. 4.5).

Patient	Absolute motor threshold (% MSO)				cPMd-iM1 connectivity measure (% unconditioned MEP)
	rMT _{AH}	aMT _{AH}	rMT _{UH}	aMT _{UH}	
1	55	47	48	45	68.9
2	55	42	38	31	65.4
3	40	34	41	37	106.3
4	52	32	37	22	120.6
5	51	43	45	37	105.9
6	41	29	40	28	61.6
7	40	35	37	32	104.4
8	42	40	46	38	91.5
9	60	40	52	36	103.8
10	82	60	50	39	118.1
11	65	44	44	30	65.0
12	100	93	52	38	122.4
Mean \pm Std	56.9 \pm 18.2	44.9 \pm 17.1	44.1 \pm 5.6	34.4 \pm 6.1	94.5 \pm 23.2

Table 4.3. Motor thresholds rMT, resting motor threshold; aMT, active motor threshold; AH, affected hemisphere; UH, unaffected hemisphere; cPMd, contralesional dorsal premotor cortex; iM1, ipsilesional motor cortex.

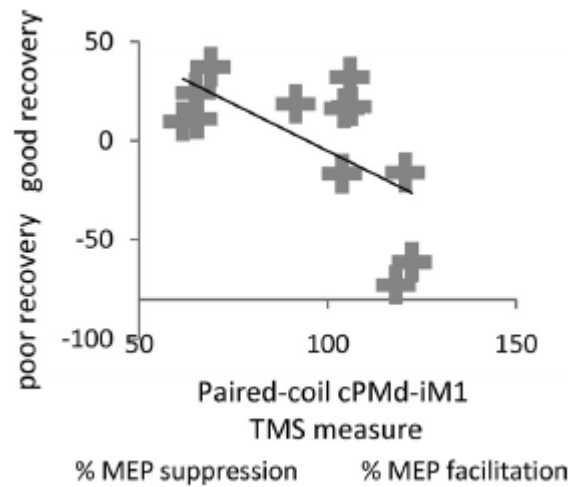


Figure 4.5. Correlation of paired coil PMd-M1 interaction with clinical score

Scatterplot showing correlation, with regression line, between the combined clinical score and the interhemispheric PMd-M1 influence measured with paired-coil TMS (conditioned MEP / unconditioned MEP as a %) in each patient. For the combined clinical score (along the y-axis) a high value indicates good residual motor function. This measure correlated with the value of the interhemispheric cPMd-iM1 influence shown along the x-axis: a better motor recovery was associated with a physiological “inhibitory” effect whereas poorer recovery was associated with less interhemispheric inhibition or even facilitation (i.e. paired-coil effects of >100%, as for the rightmost cases).

It is unlikely that our paired-coil TMS result can be explained by differences in rMTs or MEP size, as follows: (1) resting motor thresholds from either hemisphere did not correlate with the paired-coil PMd-M1 measure [rMT_{affected hemisphere}: $r = 0.36$, $p = 0.25$, not significant; rMT_{unaffected hemisphere}: $r = 0.25$, $p = 0.43$, not significant]; (2) the partial correlation of the paired-coil cPMd-iM1 measure with the combined clinical score remained significant even after accounting for rMT_{affected hemisphere} and test pulse MEP size ($r = -0.55$, $p < 0.05$); (3) mean MEP amplitudes for test pulses were not significantly different from the desired 1mV peak-to-peak amplitude (one-sample t test, $t(11) = 1.43$; $p = 0.18$), thus ruling out systematic floor or ceiling effects that might otherwise have prevented us from detecting significant inhibition or facilitation.

4.3.3 Relation between concurrent TMS-fMRI results and combined clinical score

In a separate experiment, we assessed the state-dependent influence of cPMd TMS on brain regions in either hemisphere that were activated by hand-grip with the paretic hand. First, we assessed the effects of the grip task performed with the affected hand (Fig. 4.2*b*; Table 4.4).

Anatomical Region	Side	Talairach coordinates in MNI space			Max. Z-score	Cluster P-value
		x	y	z		
Main effect of grip vs rest (irrespective of TMS)						
Postcentral gyrus	i	48	-24	35	4.72	<0.001
Supplementary motor area		3	-6	69	4.62	
Dorsal premotor cortex	i	30	-15	72	4.22	
Inferior frontal gyrus	c	-51	6	27	4.26	<0.05
Superior parietal lobule	i	24	-60	57	4.60	<0.001
Superior parietal lobule	c	-33	-48	60	5.01	<0.001
Inferior frontal gyrus	i	63	12	18	3.69	<0.05
Dorsal premotor cortex	c	-24	-12	63	3.89	<0.05
Ventral premotor cortex	c	-45	0	57	3.84	<0.05
Main effect of TMS high vs low (irrespective of TMS)						
Rolandic operculum	i	51	-24	21	3.80	<0.05
Superior temporal gyrus	c	-54	-30	12	3.92	<0.05
Middle cingulate cortex	c	-3	9	39	3.71	<0.05

Table 4.4 Locations of main effects. SPM main effect of grip vs rest (irrespective of TMS) and main effect of TMS high vs low TMS (irrespective of grip)

Height threshold of $T > 3.5$, uncorrected for multiple comparisons across whole brain, and extent (or cluster) threshold set at $P < 0.05$, corrected for multiple comparisons across whole brain. i: ipsilesional; c: contralesional

As expected, relative activity increases during grip with the affected hand compared with rest (regardless of TMS intensity) were seen in ipsilesional sensorimotor cortex

(including precentral and postcentral sulcus and extending into dorsal premotor cortex), caudal inferior frontal gyrus, bilateral middle cingulate cortex, and supplementary motor area, plus superior parietal lobule and dorsal and ventral premotor cortex, including the putative stimulation site in cPMd. Grip-related activity varied across our patient group. Those with greater impairment of the paretic hand (i.e., lower clinical scores) exhibited more activity during hand grip in secondary motor areas, consistent with previous observations (Ward et al 2003a,b). The magnitude of brain activity during affected hand grip correlated negatively with the combined clinical score in ipsilesional PMd (peak $x = 39$, $y = -12$, $z = 51$, z -score = 4.68), supplementary motor area ($x = 3$, $y = 0$, $z = 54$, z -score = 3.75), and contralesional PMd/M1 ($x = -39$, $y = -6$, $z = 48$, z -score = 3.67) (Fig. 4.6). Importantly for interpretation of our later results, we also noted a progressive posterior shift in the peak of sensorimotor cortex activation with increasing motor impairment ($r = 0.59$, $p = 0.043$) (see Fig. 4.8 below), as previously described (Rossini et al 1998; Pineiro et al 2001; Cramer 2004; Cramer & Crafton 2006).

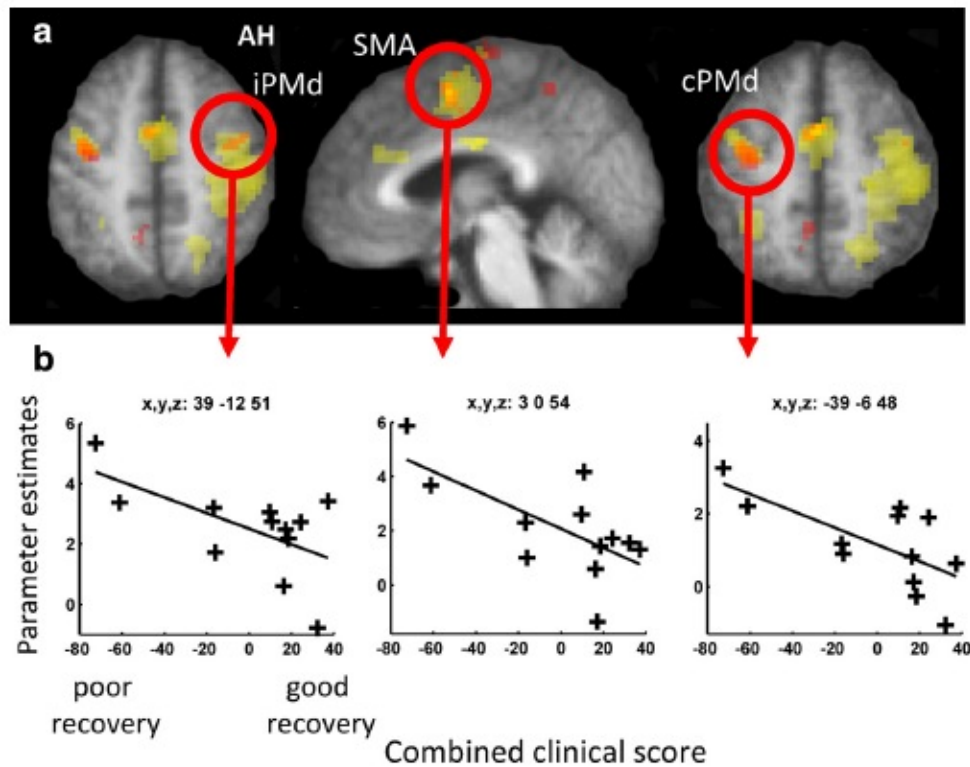


Figure 4.6. Grip-related fMRI activity correlations with residual motor function. *a*, SPM for the main effect of hand grip (minus rest) is shown in yellow, overlaid onto the mean normalized T1-weighted structural image from all participants. Activation clusters in which a significant relationship between hand grip-related activity and the combined clinical score was observed are shown in orange ($p < 0.05$, corrected, for multiple comparisons across the brain). *b*, Parameter estimates from each individual patient for the main effect of hand grip (minus rest; shown along the y-axis) plotted against the combined clinical score from each patient (along the x-axis) for the circled regions in *a*, at the coordinates listed. iPMd, Ipsilesional PMd; SMA, supplementary motor area; AH, affected hemisphere.

Second, we compared all events with high-intensity TMS to those with low-intensity TMS, finding relative increases in BOLD signal in middle cingulate cortex as well as auditory cortex bilaterally, presumably because of the somewhat louder click associated with higher intensity TMS (Table 4.4) (Hanakawa et al 2009; Siebner et al 1999; Bestmann et al 2004; Baudewig et al 2001; Bohning et al 1998).

Third, we examined the critical interaction of TMS intensity and motor state (i.e. hand grip vs rest) using the contrast $TMS_{high} (grip-rest) > TMS_{low} (grip-rest)$. The resulting voxelwise parameter estimates from this contrast reflect the magnitude of the influence of the stimulated cPMd on other brain regions during hand grip compared with rest. For instance, if cPMd has no influence over region A then there will be no difference between grip minus rest for the high- versus low-intensity TMS conditions in the voxels corresponding to region A. If, however, there is an influence of cPMd TMS on region A that increases during hand grip then the high-intensity TMS condition will lead to a local increase in BOLD signal in region A during hand-grip, and thus the interaction contrast $TMS_{high} (grip-rest) > TMS_{high} (grip-rest)$ will be positive. Although we found no consistent effects for the patient group on average, based on previous work (Ward et al 2003a,b), our expectation was to find variability in relation to the degree of impairment. We therefore tested whether the influence of cPMd TMS on other brain regions [a more facilitatory influence being reflected in a higher value for the contrast $TMS_{high} (grip-rest) \times TMS_{high} (grip-rest)$] correlated with the combined clinical score for each patient (lower value reflecting greater motor impairment). We found that in our patients with greater clinical impairment, there was a more facilitatory effect of cPMd on only one region in

the ipsilesional hemisphere. The most significant voxel was posterior and ventral to ipsilesional hand area of M1 (peak at $x = 51, y = -30, z = 42$) (Fig. 4.7).

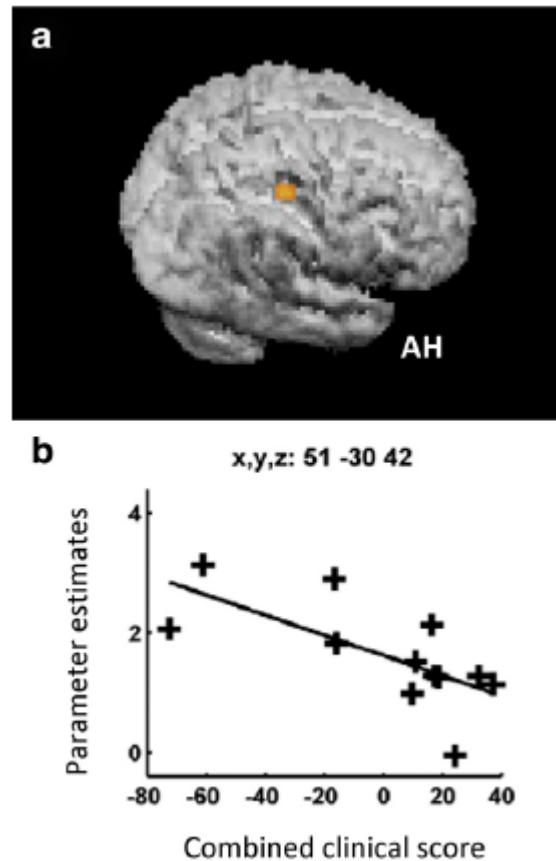


Figure 4.7. Relating remote influence of PMd to clinical scores

a, SPM for the interaction term $TMS_{high} (grip-rest) > TMS_{low} (grip-rest)$ overlaid on the rendered mean structural scan from all patients. The influence of cPMd on this cluster [assessed by the parameter estimates for $TMS_{high} (grip-rest) > TMS_{low} (grip-rest)$] are plotted against the combined clinical score for each patient. AH, Affected hemisphere. **b**, The facilitatory influence of contralesional PMd during hand grip (as measured with concurrent TMS-fMRI) correlated with combined clinical score in this ipsilesional cluster extending across posterior sensorimotor cortical regions.

At a lower threshold ($p < 0.005$), the significant cluster extended between $y = -18$ and -38 in the anterior–posterior direction and between $z = 38$ and 58 in the dorsal–ventral direction, overlapping greatly with the sensorimotor cortex activation seen in our patients for the grip task overall. We consider this region to be part of the sensorimotor cortex in our patients, particularly since we have already observed that hand grip-related activity was located progressively more posteriorly in our more impaired patients (Fig. 4.8).

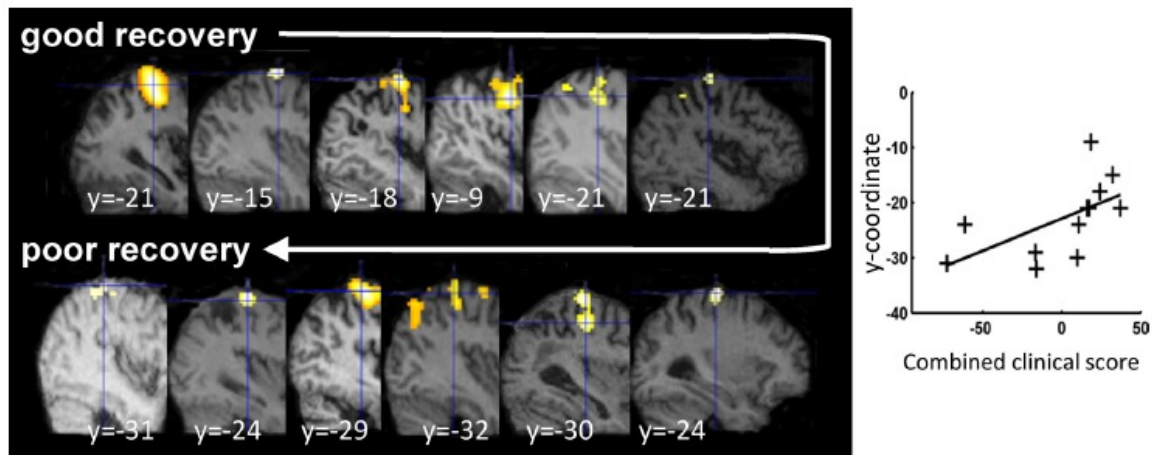


Figure 4.8. Posterior shift in motor cortical activation

The relationship between the ipsilesional peak hand grip-related signal change and the combined clinical score is shown. The SPM for the main effect of hand grip is overlaid on the individual structural scan of each patient. A posterior shift of activity in ipsilesional cortex was observed, with less well recovered patients exhibiting progressively more posterior peak activity. The y -coordinate from each individual patient for the peak activity for the main effect of grip versus rest (shown along the y -axis) is plotted against the combined clinical score from each patient (along the x -axis).

Contralesional PMd TMS did not exert a significant influence on any other brain region during hand grip compared with rest, neither on average nor when correlated with impairment. No effects in hand grip-related regions were observed for the negative interaction (i.e. the reverse SPM contrast).

4.3.4 Relation between concurrent TMS-fMRI results and separate interhemispheric paired-coil TMS physiological results

We were also specifically interested in how patient-by-patient variability in our TMS paired-coil cPMd-iM1 measure may explain any variability in our fMRI results, over and above variability already explained by the main effect of hand grip and the TMS_{high} (grip–rest) > TMS_{low} (grip–rest) interaction. We found that a less inhibitory / more facilitatory influence of cPMd on iM1 in the paired-coil TMS experiment correlated positively with the magnitude of BOLD signal changes during hand grip (regardless of TMS intensity) in cPMd ($x = -30, y = -12, z = 63, z\text{-score} = 3.98, p < 0.05$) (Fig. 4.9).

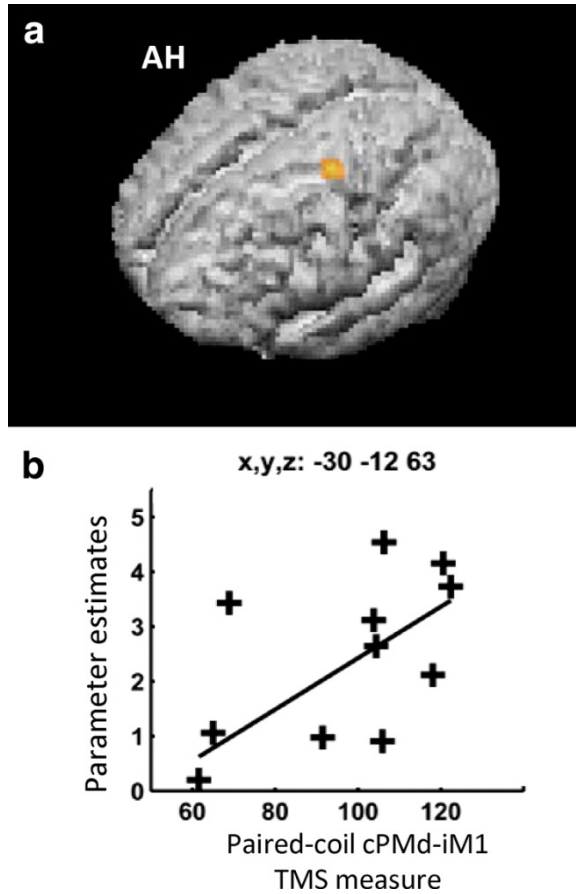


Figure 4.9. Relating grip-related activity to PMd-M1 interaction

a, Parameter estimates for the main effect of hand grip minus rest (shown along the y-axis) are plotted in a patient-by-patient manner against the interhemispheric cPMd-iM1 influence (along the x-axis). AH, Affected hemisphere. **b**, cPMd-iM1 interhemispheric influences at rest (as revealed by paired coil TMS) correlated with hand grip-related activity in contralesional premotor cortex. Hand grip-related activity at the identified stimulation site in cPMd correlated with the separately measured interhemispheric paired-coil cPMd-iM1 influence ($p < 0.05$, corrected, for multiple comparisons across the brain). The SPM for this effect is projected onto the rendered, average, normalized, T1-weighted structural image from all participants.

Second, the TMS paired-coil cPMd-iM1 measure correlated positively with the parameter estimates from the TMS_{high} (grip–rest) > TMS_{low} (grip–rest) interaction in only one region, ipsilesional inferior central sulcus ($x = 36, y = -12, z = 45$) corresponding to Brodmann area 4p, the posterior part of primary motor cortex (Fig. 4.10).

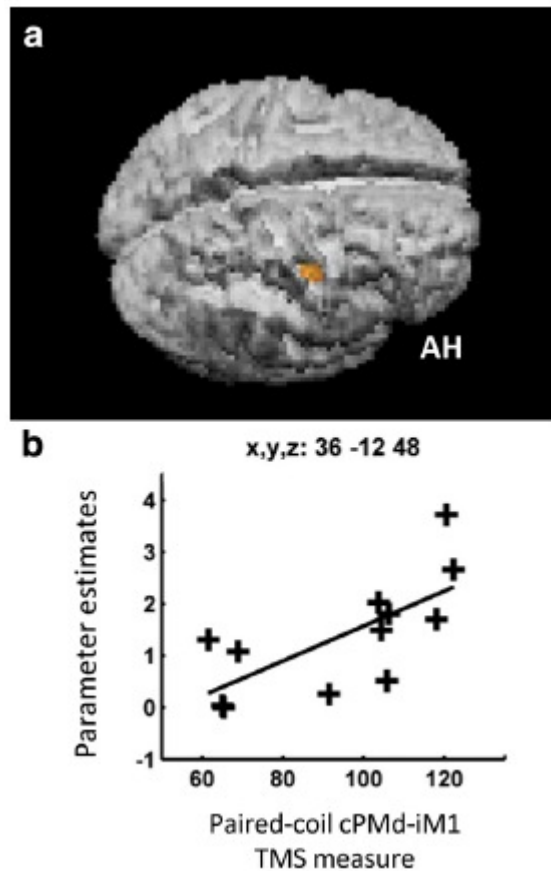


Figure 4.10. Relating remote influence of PMd to PMd-M1 interaction

Brain regions in which the influence of cPMd during hand grip (as measured with concurrent TMS-fMRI) was greater when cPMd had a less inhibitory/more facilitatory affect on ipsilesional M1 (as measured with paired-coil TMS). **a**, The SPM for the correlation seen in ipsilesional posterior central sulcus, BA4p, is overlaid on the rendered mean structural scan from all patients. **b**, Each patient's parameter estimate for the interaction term TMS_{high}

(grip– rest) > TMS_{low} (grip–rest) in ipsilesional posterior central sulcus, BA4p, plotted against the paired-coil measure of the interhemispheric cPMd-iM1 influence. AH, Affected hemisphere.

Thus patients with more pathological cPMd-iM1 paired-coil results showed stronger interaction effects of cPMd TMS in this posterior ipsilesional motor region.

For completeness, in an additional analysis we also looked for any age-dependent changes in motor function by additionally including age as covariates of no interest at the second level of analysis. No influences of age or time-after-stroke were observed for the critical contrasts in any hand grip-related areas (data not shown).

4.4 Discussion

Contralesional PMd is thought to contribute to the support of recovered motor function after stroke, more so in patients with greater impairment (Johansen-Berg et al 2002; Lotze et al 2006; Ward et al 2007), but the mechanism by which it exerts this influence has remained unknown. Work in primates has demonstrated that direct descending projections from secondary motor regions, including PMd, have longer latencies and are weaker than those from M1 (Boudrias et al 2010), suggesting additional synapses in the anatomical pathway for their actions on motoneurons. The descending motor pathway from PMd could involve the intermediate zone of the spinal cord or even propriospinal premotoneurons (Mazevet et al 2003; Stinear & Byblow 2004), which receive projections from premotor cortex, at least in nonhuman primates (Benecke et al 1991). PMd is also reciprocally connected with ipsilateral (Lu et al 1994; Wise et al 1997; Dum & Strick 2005) and contralateral (Marconi et al 2003) cortical motor areas, including M1. An

alternative route through which cPMd can influence residual motor function might thus be via corticocortical pathways. Our results provide evidence that in patients with more impairment, cPMd exerts an increasing influence on surviving sensorimotor cortex in the ipsilesional hemisphere. We did not find any evidence for correspondingly increased influences on any other brain regions, including other secondary motor areas.

In the current experiments, across our group of stroke patients, variations in the influence of cPMd on surviving brain regions relate not only to clinical motor scores, but also to separate neurophysiological (paired-pulse TMS) markers of interhemispheric interactions. The magnitude of the influence of cPMd TMS upon BOLD signal in a posterior part of the ipsilesional sensorimotor cortical region was greater in those patients with more clinical motor impairment. Since clinical scores may not capture all of the important variability between patients (Talelli et al 2008), we additionally used an independent physiological (paired-pulse) measure of cPMd-iM1 influence to further interrogate our fMRI results. We found that a significant proportion of the variability in the influence of cPMd TMS on BOLD signal in another ipsilesional posterior sensorimotor region, BA4p, was accounted for by this separate electrophysiological cPMd-iM1 effect.

One possible explanation of our results is that hand grip related activity in cPMd may increase excitability in ipsilesional sensorimotor regions and thereby facilitate an increase in the gain of descending motor signals to the affected upper limb. This influence could become more important in patients with greater impairment, since cPMd is more useful

for motor performance in more impaired patients (Johansen Berg et al 2002). As the hand region of M1 becomes less important in motor control with increasing corticospinal tract damage (Ward et al 2007), an increased influence on that part of disconnected M1 might not enhance motor output in more impaired patients. However, more posterior parts of sensorimotor cortex may retain their direct projections to spinal cord motoneurons in some patients and hence may provide a better target for cPMd to influence motor output. Posterior shifts in the peak of sensorimotor cortex activation have been previously observed in stroke patients, presumably as a consequence of effective (or partial) disconnection of the hand area from the corticospinal tract (Pineiro et al 2001). Corresponding posterior shifts in the TMS motor hot spot (Rossini et al 1998) suggest that these more posterior regions are intimately involved in motor output to the affected hand. Activity during affected hand grip was indeed found more posteriorly with greater impairment in our own patient cohort (Fig. 4.8).

Another cortical motor region known to be more active in patients with greater impairment and/or more corticospinal tract damage is Brodmann area (BA) 4p (Ward et al 2003a, 2006), in the deep part of the anterior bank of central sulcus. Here we found that cPMd TMS exerted greater grip-related influence on BA4p in those patients for whom the paired-coil cPMd-M1 measure was most abnormal. By using both a clinical and neurophysiological measure as covariates in our fMRI analysis, we were able to identify two regions of the ipsilesional grip-related network via which the influence of cPMd increases in more impaired patients. This indicates changes in interregional influence within the motor network that relate to the impairment of corticospinal system

function. A question for future research is how this reorganised inter-regional influence arises, including whether it is an inevitable consequence of corticospinal disruption by the subcortical lesions themselves or whether it can also be shaped by some forms of physical therapy or motor practice. Answering this question will require longitudinal studies that make specific therapeutic or practice manipulations.

More generally, the present concurrent TMS-fMRI approach can highlight state-dependent interactions between remote but interconnected regions across the brain (Bestmann et al 2008b; Ruff et al 2006; Sack et al 2007). Concurrent TMS-fMRI can provide a new type of additional formation compared with paired-pulse double-coil approaches alone or purely correlative fMRI approaches without the causal TMS intervention. Relating BOLD signal changes directly to the physiological changes evoked by TMS inevitably demands the combination of more invasive recordings of neural activity with fMRI. The relationship between the observed BOLD signal changes reported here and the physiological inhibition or facilitation observed in our paired coil TMS experiment must therefore be considered with care. However, recent work has clearly demonstrated how the concurrent TMS-fMRI approach can be applied to highlight differences in TMS evoked activity changes locally and in interconnected regions (Bestmann et al 2008b; Driver et al 2009), including for pathological conditions such as depression (Li et al 2004). We show how one can apply this technique to study state-dependent changes in interregional influences following stroke and how such changes relate to individual clinical and electrophysiological markers of residual motor function. Our present results relate only to the state-dependent influence of cPMd on other brain

regions, but the present approach could now be used to study the influence of other cortical motor regions on surviving motor networks after stroke.

In general, interhemispheric PMd-M1 influences studied with paired-coil TMS can be inhibitory or facilitatory, depending on the exact conditioning intensity used (Baumer et al 2006). We have taken our results to indicate a shift in the balance toward net facilitation in the more impaired patients. The implication is that input from cPMd might now assist ipsilesional brain regions to produce movement. The fact that transient interference with the activity of cPMd using single-pulse TMS impairs movement of the paretic hand in stroke patients (Johansen-Berg et al 2002) appears consistent with such a change to a facilitatory role. Interestingly, a different argument has been made for the possible role of cM1 (Hummel & Cohen 2006). Using paired coil TMS, Murase et al (2004) found that, unlike healthy controls, stroke patients with unilateral motor deficits showed abnormally increased interhemispheric inhibition from cM1 before movement of the affected hand. They proposed that persisting interhemispheric inhibition from cM1 could interfere with movement controlled by the damaged hemisphere, contributing to motor impairment. Indeed, this reasoning led to a potential treatment approach in stroke: using low-frequency rTMS or cathodal transcranial direct-current stimulation, with the aim of reducing activity of cM1 and thereby promoting greater function in iM1 (for review, see Hummel & Cohen 2006; Talelli & Rothwell 2006). It is unclear why input from cM1 should interfere with functioning of the damaged hemisphere, which appears to be facilitated by input from cPMd. One possibility is that control of inhibition from cM1 is normally managed by circuits in iM1 that suppress inputs before movement. If these

are damaged by stroke, then the influence of cM1 will appear negative. Conversely, inputs from cPMd may normally assist production of certain types of movement (O'Shea et al 2007a,b) and this facilitation may increase after damage to the lesioned hemisphere. Although the physiological signatures for cM1 and cPMd influences on iM1 appear very different, the commonality for both sets of observations is that interhemispheric influences from contralesional to ipsilesional motor regions, as assessed with paired-coil measures, are systematically more abnormal in patients with more impaired clinical motor function.

In conclusion, our results indicate that contralesional PMd exerts a causal influence on ipsilesional sensorimotor regions that are active during movement of the weak hand that increases with impairment. Although previous work led to the hypothesis that cPMd is important for supporting recovered motor function, particularly in more impaired patients, here we were able to show for the first time via concurrent TMS-fMRI that the mechanism of this support is likely to be a remote, state dependent influence on ipsilesional sensorimotor cortex that is stronger during grip with the paretic hand, more so in more impaired patients. Furthermore, physiological changes in interhemispheric cortical influences can explain unique aspects of motor system activity in stroke patients using their affected hand. More generally, this work highlights an important property of the CNS in that the functional influences of brain regions upon others are adaptable in clinically and behaviourally relevant ways.

Chapter 5

The effects of Theta Burst Stimulation on corticospinal excitability and subsequent motor task acquisition: neuromodulation of observed effects using drugs

Work described in this chapter was published in Clinical Neurophysiology and Cerebral Cortex:

Swayne OB, Teo JT, Greenwood RJ, Rothwell JC. The facilitatory effects of intermittent theta burst stimulation on corticospinal excitability are enhanced by nicotine. Clin Neurophysiol. 2009 Aug;120(8):1610-5.

Teo JTH*, Swayne OB*, Cheeran B, Greenwood RJ, Rothwell JC. Human theta burst stimulation enhances subsequent motor learning and increases performance variability. Cerebral Cortex 2010 Dec 1. [Epub ahead of print]

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Outline of experiments

This chapter examines the influence of pharmacological neuromodulation on the effects of Theta Burst Stimulation of the motor cortex. It is divided into 2 sections, investigating effects on corticospinal excitability (5.1) and motor behaviour (5.2) as follows:

5.1 Effects of iTBS and neuromodulation on corticospinal excitability

- Effects of nicotine, amphetamine and levodopa (Experiment 1)

5.2 Effects of iTBS and neuromodulation on motor learning

- Motor task acquisition after TBS and interaction with nicotine
(Experiment 2)
- Computer model of task acquisition
- Effect of TBS on directional variability (Experiment 3)

5.1 Effects of iTBS and neuromodulation on corticospinal excitability

5.1.1 Introduction

Intermittent theta burst stimulation (iTBS) is a form of repetitive transcranial magnetic stimulation (rTMS) that is now widely used by investigators to bring about a transient facilitation of excitability in the human primary motor cortex (Huang et al 2005). There is evidence to suggest that this form of stimulation may induce NMDA-dependent synaptic strengthening similar to long term potentiation (LTP) (Huang et al 2007; Teo et al 2007). While it is known that other forms of plasticity induced by non-invasive stimulation (Kuo et al 2007) or by motor practice (Meintzschel et al 2006) may be pharmacologically enhanced this has never been demonstrated for iTBS. Apart from the mechanistic information that such an approach may provide, a means of enhancing the effects of iTBS

would be of interest to investigators seeking more effective ways of modulating cortical excitability.

Cholinergic modulation of synaptic plasticity has been well documented. In animal models, acetylcholine receptor blockade inhibits LTP in layer II/III synapses in the primary motor cortex (Hess & Donoghue 1999). Moreover, activation of nicotinic acetylcholine receptors can both modulate NMDA-dependent synaptic plasticity (Ji et al 2001; Ge and Dani 2005) and can also induce LTP independently of the NMDA receptor (Matsuyama et al 2000; Yamazaki et al 2005). As a short term change in synaptic strength is thought to mediate at least some of the excitability increase resulting from iTBS nicotinic modulation would therefore seem to be a reasonable candidate in an attempt to enhance its effects.

Dopaminergic modulation of plasticity protocols has been demonstrated previously in the context of transcranial direct current stimulation (tDCS) of the human motor cortex (Kuo et al 2008a), and use-dependent plasticity (Meintzschel & Ziemann 2006). It is suggested that dopaminergic stimulation may enhance the response to such protocols in a dose-dependent manner (Kuo et al 2008a) and may promote synaptic changes resulting from LTP (Molina-Luna et al 2009). Dextro-amphetamine provides stimulation of both the dopaminergic and noradrenergic neurotransmitter systems. Amphetamine is known to prolong the effects of anodal tDCS (Nitsche et al 2004b), and the induction of use-dependent plasticity is enhanced by noradrenergic stimulation (Meintzschel & Ziemann 2006), which has also demonstrated beneficial effects on motor recovery following stroke

in animal models. The interactions of levodopa and dextro-amphetamine with iTBS are therefore also of interest.

We set out to test the interactions of these neurotransmitter systems with the effects of iTBS. We hypothesised that if changes in synaptic strength contribute to the facilitatory effects of iTBS on corticospinal excitability then we might expect these effects to be greater in the presence of these neuromodulators.

5.1.2 Experiment 1 Methods

We tested the effects of priming the primary motor cortex with single oral dose of nicotine (Experiment 1A), or of either levodopa or dextro-amphetamine (Experiment 1B) before delivering iTBS in 2 separate randomised, placebo-controlled cross-over design studies.

5.1.2.1 Subjects

Healthy right-handed subjects participated in these experiments. Subjects who smoked, or had smoked within the previous year, were excluded from the study and no subjects were taking either short-term or long-term medication. Ten subjects participated in the nicotine experiment, Experiment 1A (3 female; mean age 29.6 ± 4.7 SD). Ten subjects participated in the dopamine / dextro-amphetamine experiment, Experiment 1B (4 female; mean age 32 ± 4.7 SD). Of these, all participated in the amphetamine arm while six participated in the dopamine arm (1 female; mean age 30 ± 4.5 SD).

5.1.2.2 Medication

For Experiment 1A, subjects were given either 2 mint-flavoured 2 mg nicotine lozenges (active medication) or 2 inactive mint lozenges (placebo). We chose lozenges in order to achieve rapid nicotine absorption, with maximum plasma levels reached in 60 minutes (Hukkanen et al 2005; Russell et al 1987; Tobacco Advisory Group 2000). In order to further disguise any taste associated with nicotine, subjects were also given a strong-tasting menthol lozenge (Fisherman's Friend) in both conditions. Subjects were asked not to chew these lozenges, but to keep them in the mouth until fully dissolved. For Experiment 1B, subjects were given tablets prepared by the National Hospital for Neurology and Neurosurgery pharmacy containing either dextro-amphetamine 10 mg, ascorbic acid 100 mg or levodopa / carbidopa in the form of Madopar 100/25 mg. Both investigator and subjects were blinded to the drug taken during each session.

5.1.2.3 Theta Burst Stimulation

Stimulation was delivered using a Magstim Rapid stimulator (Magstim Co., Dyfed, UK) connected to a figure-of-eight coil with an internal wing diameter of 70 mm, held with the handle pointing posterolaterally. Electromyographic (EMG) recordings were made using a belly-to-tendon montage from the left first dorsal interosseous muscle (FDI). The raw signal was amplified and filtered with a band-pass filter of 30 Hz to 1 kHz (Digitimer Ltd). Signals were digitized at 2 kHz (CED Power1401, Cambridge Electronic Design, Cambridge, UK) and stored on a laboratory computer for offline analysis. The location of the hand representation in the right hemisphere was determined, defined as the position at which stimulation produced optimal MEPs in the left FDI. The active motor threshold

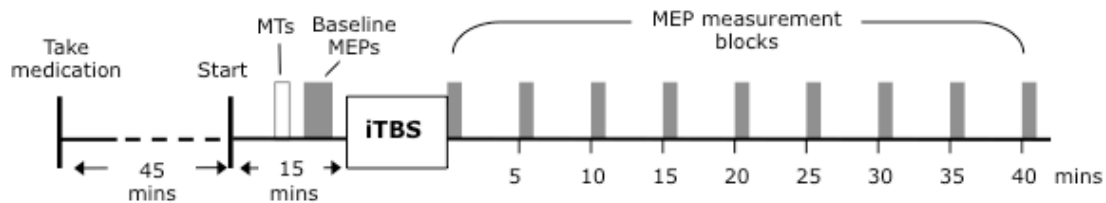
(aMT) was assessed during voluntary contraction of the target FDI at approximately 10% of maximum force, and was defined as the lowest stimulus intensity required to evoke an MEP of >200 μ V in 5 out of 10 trials. Theta Burst Stimulation was given according to the intermittent (iTBS) protocol described by Huang et al. (2005). Bursts consisting of 3 pulses at 50 Hz, at an intensity of 80% aMT, were repeated every 200 ms (ie 5 Hz) for 2 seconds. This 2 second train was repeated once every 10 seconds for 20 repetitions, a total of 193 seconds. This stimulation protocol has been shown to produce an increase in corticospinal excitability lasting up to 15 minutes (Huang et al 2005). For sham stimulation, the coil was held rotated 90 degrees so that the point of contact with the scalp was unchanged but the handle pointed vertically upwards.

5.1.2.4 Study design

The experiments employed a randomised, double-blinded, placebo-controlled cross-over study design. For Experiment 1A (nicotine / placebo) subjects attended 2 sessions whose order was counter-balanced across the group: (1) iTBS + Nicotine; and (2) iTBS + placebo. Nine of these subjects also participated in a control experiment investigating the effects of nicotine in the absence of iTBS. For Experiment 1B subjects attended either 2 (four subjects) or 3 (six subjects) sessions, consisting of iTBS + (dextro-amphetamine / levodopa / placebo). For each subject, sessions were separated by at least a week. For Experiment 1A the experimental session started 45 minutes after ingestion of the medication / placebo, so that iTBS was delivered at 1 hour, coinciding with maximum plasma levels. For Experiment 1B, the interval between medication and the experimental session depended on the medication taken (but was unknown to the investigator). For

Dextro-amphetamine we employed an interval of 2 hours before the start of the session, consistent with previous investigations demonstrating enhanced motor performance in stroke patients (Crisostomo et al 1988; Walker-Batson et al 1995): iTBS was therefore delivered at 2 hours and 15 minutes. Levodopa iTBS was delivered at 1 hour, coinciding with peak plasma levels and physiological effect (Floel et al 2005b; Kuo et al 2008a). An interval of 90 minutes was used for placebo. The experimental outline is shown in Figure 5.1.1.

Experiment 1A



Experiment 1B

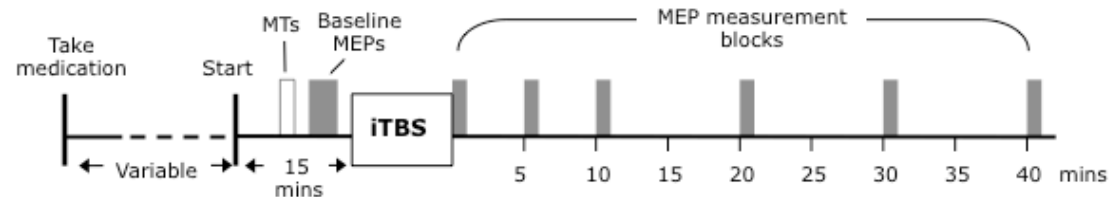


Figure 5.1.1. Experiment 1 outline

Please see text for timings of start of session in Experiment 1B

Subjects sat comfortably in a chair with both arms resting on a pillow and the hand representation of the right motor cortex was located as described. The measure of corticospinal excitability used here was the amplitude of motor evoked potentials (MEPs) elicited in response to single pulse TMS. These were recorded in blocks of 10 trials at 0.2 Hz using a Magstim 200 monophasic stimulator connected to a figure-of-eight coil

(internal wing diameter 70 mm). Before iTBS, 2 baseline blocks were recorded (total 20 trials) using a stimulus intensity adjusted to evoke an MEP of 1 mV amplitude in the left FDI, which was kept relaxed throughout: this stimulus intensity was used for the remainder of the session. iTBS was given as described above, starting at the appropriate interval after the medication was taken (see above). An MEP block was recorded immediately following the end of iTBS (10 trials). For Experiment 1A, subsequent blocks were recorded every 5 minutes up to 40 minutes. For Experiment 1B, subsequent blocks were recorded at 5, 10, 20, 30 and 40 minutes. In Experiment 1A (nicotine) subjects also returned for a control experiment (not blinded), performed to test whether nicotine on its own modulates corticospinal excitability: subjects were given the nicotine lozenges and MEP blocks were recorded at the same time points but no iTBS was given. For both the main and control experiments subjects were unaware of the scientific hypotheses being tested.

5.1.2.5 Data analysis

Individual trials were examined offline and any with EMG activity prior to the TMS stimulus artifact were discarded. Peak-to-peak MEP amplitudes were measured in the remaining trials. Mean MEP amplitudes were determined for each time point and in each subject and were expressed as logarithm base 10 to reduce skew. In Experiment 1A (nicotine) it was necessary to reduce the number of time points for statistical comparison in order to leave sufficient residual degrees of freedom for repeated measures ANOVA. Therefore adjacent time points were combined by taking a mean, so that values were obtained for a total of 6 time epochs (baseline, 0-5, 10-15, 20-25, 30-35 and 40 minutes).

Repeated-measures ANOVA was used to test for the effects of ‘time’ (or ‘block’), ‘preparation’ and ‘stimulation’, and their interactions. Post-hoc paired t-tests were used to examine differences from baseline (uncorrected for multiple comparisons) and differences between values at individual time points. The SPSS 12.0 package was used for performing statistical analysis.

5.1.3 Results

5.1.3.1 Experiment 1A: iTBS and nicotine

Two subjects reported mild transient nausea after taking nicotine. There were otherwise no side-effects resulting from the medication or from iTBS. Subjects correctly identified the preparation they had taken in 80% of nicotine sessions and 80% of placebo sessions: this is significantly different from chance (Fisher’s exact test, $p=0.023$), implying that blinding was ineffective. Motor thresholds, stimulation intensities and baseline MEP amplitudes are given in Table 5.1.1 (including those for Experiment 1B). There were no differences between nicotine and placebo sessions with respect to active motor threshold (paired t-test, $P=0.519$), test stimulus intensity ($P=0.372$), iTBS stimulus intensity ($P=0.519$) or baseline MEP amplitude ($P=0.433$).

Experiment	Condition	Active Motor Threshold (%MSO)	Test Stimulus Intensity (%MSO)	iTBS Stimulus Intensity (%MSO)	Baseline MEP amplitude (mV)
1A: Nicotine	iTBS-Placebo	47.1 ± 2.9	58.1 ± 3.7	37.7 ± 2.3	0.92 ± 0.14
	iTBS-Nicotine	48.3 ± 1.9	55.4 ± 3.3	38.6 ± 1.5	1.11 ± 0.22
	No iTBS-Nicotine (Control experiment)	-	48.4 ± 3.5	-	1.02 ± 0.11
1B: D-Amphetamine	iTBS-Placebo	45.6 ± 3.0	47.5 ± 3.9	36.4 ± 2.4	1.08 ± 0.11
	iTBS-Amphetamine	45.6 ± 3.1	45.2 ± 3.6	36.6 ± 2.4	1.07 ± 0.13
1B: Levodopa	iTBS-Placebo	46.0 ± 3.4	47.5 ± 2.6	36.8 ± 2.6	1.11 ± 0.09
	iTBS-Dopamine	43.5 ± 3.4	44.8 ± 3.1	34.8 ± 2.7	1.19 ± 0.14

MSO, Mean stimulator output; iTBS, intermittent theta burst stimulation. Values given as Mean ± SE.

Table 5.1.1 Experiment 1: baseline physiological parameters

MEP amplitudes after iTBS are shown in a representative subject and for the group in Figures 5.1.2 and 5.1.3 respectively.

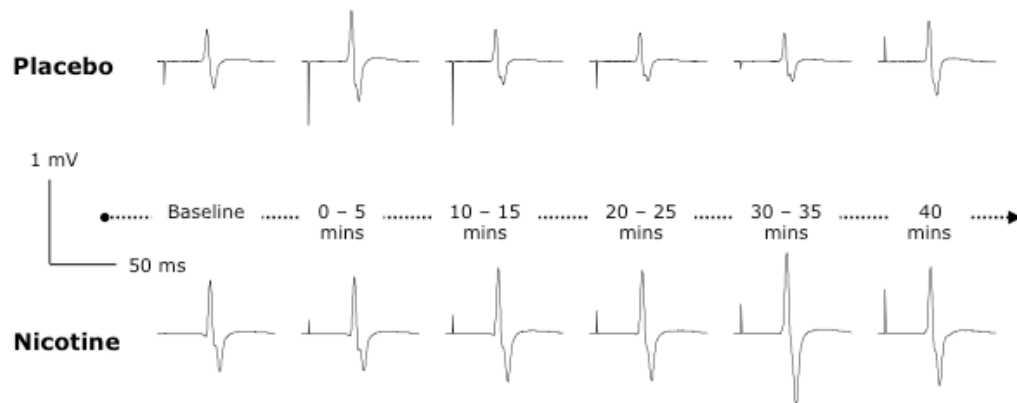


Figure 5.1.2. MEP amplitudes in an individual subject

Data are shown for one representative subject as averaged MEPs at each time epoch. The times shown are in minutes following the end of iTBS to the motor cortex. In the placebo condition, there is an increase in MEP amplitudes at 0-5 minutes, before excitability returns to baseline levels. In the nicotine arm, there is a more pronounced increase in MEP amplitudes that starts later and is more prolonged.

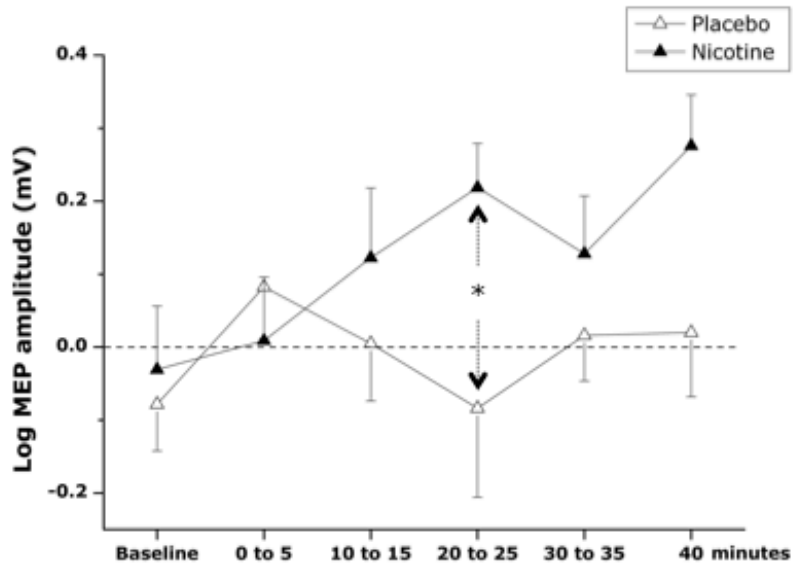


Figure 5.1.3. MEP amplitudes for the group

Group data is shown, as mean \pm standard error. The effect of time differed in the 2 experimental arms (significant ‘Time’ x ‘Preparation’ interaction – see text for details). In the placebo arm, MEP amplitudes were significantly increased in comparison to baseline at 0-5 minutes before returning to normal. In the nicotine arm there was a more pronounced increase in MEP amplitudes that started later and was more prolonged, being significant at the last 3 time points (see text for t-tests). MEPs were significantly larger in the nicotine arm than the placebo arm at 20-25 minutes (paired t-test, $P=0.042$).

After iTBS, a 2-way ANOVA with main factors ‘Time’ and ‘Preparation’ revealed differing effects of time on MEP amplitudes in the nicotine and placebo arms (‘Time’ x ‘Preparation’ interaction, $F_{5,5}=78.7$, $P<0.001$; main effect of ‘Time’, $F_{5,5}=2.6$, $P=0.159$; main effect of ‘Preparation’, $F_{1,9}=8.5$, $P=0.017$). Post-hoc t-tests revealed that with placebo MEP amplitudes were greater than baseline 0-5 minutes after iTBS (paired t-test, $P=0.038$) but not at later time points. With nicotine, by contrast, MEP amplitudes were

unchanged at earlier time points but were raised at 20-25, 30-35 and 40 minutes ($P=0.034$, $P=0.019$ and $P=0.023$ respectively). At the 20-25 minute time point MEPs were significantly larger with nicotine than with placebo ($P=0.042$). Thus the excitability increase following iTBS was greater at later time points with nicotine, starting later (20 minutes) and lasting longer (still present at 40 minutes).

5.1.3.2 Experiment 1A: control experiment

The possibility that nicotine itself may have caused the increase in MEP amplitudes was investigated in a control experiment ($N=9$), in which nicotine was given but iTBS was omitted (Figure 5.1.4). Conditions were identical other than the omission of iTBS.

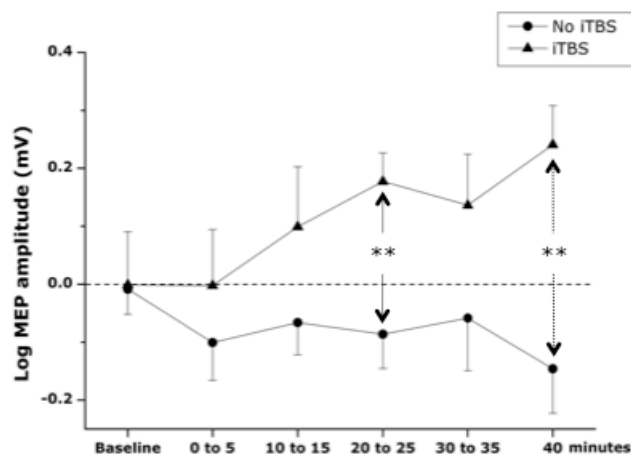


Figure 5.1.4. Effect of nicotine alone (without iTBS)

No increase in excitability was observed with nicotine alone. The effect of time differed in the 2 experimental arms (significant ‘Time’ x ‘Preparation’ interaction – see text for details). MEP amplitudes were significantly greater with iTBS at 20-25 minutes and 40 minutes (paired t-tests, $P=0.006$ and $P<0.001$ respectively).

MEP amplitudes in the ‘Nicotine alone’ and ‘Nicotine + iTBS’ conditions were compared using a 2-way ANOVA with main factors ‘Time’ and ‘Stimulation’. The effects of time differed in these 2 conditions (‘Time’ x ‘Stimulation’ interaction, $F_{5,4}=16.0$, $P=0.009$; main effect of ‘Time’, $F_{5,4}=0.7$; $P=0.634$; main effect of ‘Stimulation’, $F_{1,8}=6.8$, $P=0.032$), and post-hoc t-tests revealed that MEP amplitudes with nicotine alone never significantly differed from baseline ($P>0.05$ at all time points). MEPs were significantly greater with stimulation than without at the 20-25 and 40 minute time points ($P=0.006$ and $P<0.001$ respectively). These results suggest that the prolonged excitability increase observed with nicotine and iTBS did not result from the nicotine alone but rather from an interaction between these 2 interventions.

5.1.3.3 Experiment 1B: dextro-amphetamine and levodopa

No subjects suffered any side-effects or adverse events from these medications. Subjects were able to identify accurately when amphetamine was taken (70% accuracy) due to a reported subjective feeling of euphoria. Subjects were also able to identify above chance which of levodopa or placebo were taken (L-DOPA at 67% accuracy, placebo at 60% accuracy). There were no differences in baseline aMT, MEP amplitude or test stimulus intensity between conditions (see Table 5.1.1 above; $p>0.05$ with student’s paired t-tests for all instances).

Fig 5.1.5 shows the after-effects of iTBS in the amphetamine and the placebo arms of the study for 10 subjects. There were no significant differences between both arms of the

experiment as analysed by a two-factorial ANOVA with factors ‘preparation’ and ‘time’ ($p>0.05$ for ‘preparation’ effect, ‘time’ effect and ‘preparation’ x ‘time’ interaction).

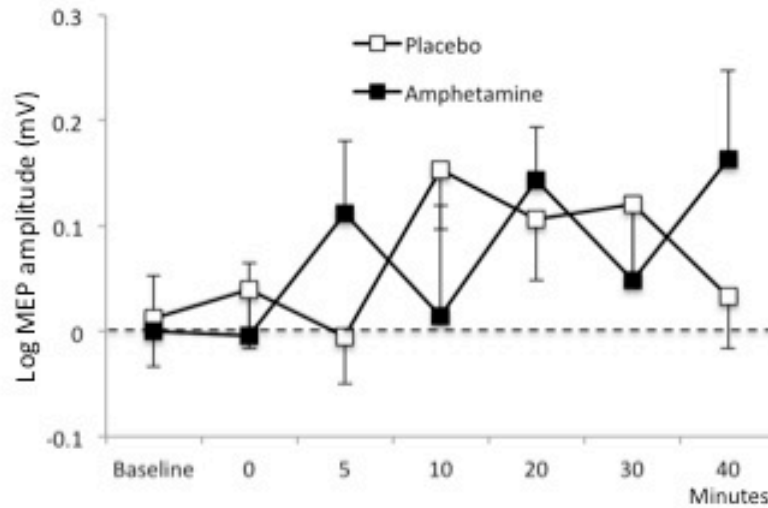


Figure 5.1.5. Effect of dextro-amphetamine

The time course of the MEP amplitudes after iTBS in the placebo (squares) and amphetamine (filled triangles) arms of the study. Errors bars represent standard error of mean. Data for 10 subjects are shown.

Fig 5.1.6 shows the after-effects of iTBS in the levodopa and placebo arms of the study for 6 subjects. Again, there were no significant differences between both arms of the experiment as analysed by a two-factorial ANOVA with factors ‘preparation’ and ‘time’ ($p>0.05$ for ‘preparation’ effect, ‘time’ effect and ‘preparation’ x ‘time’ interaction).

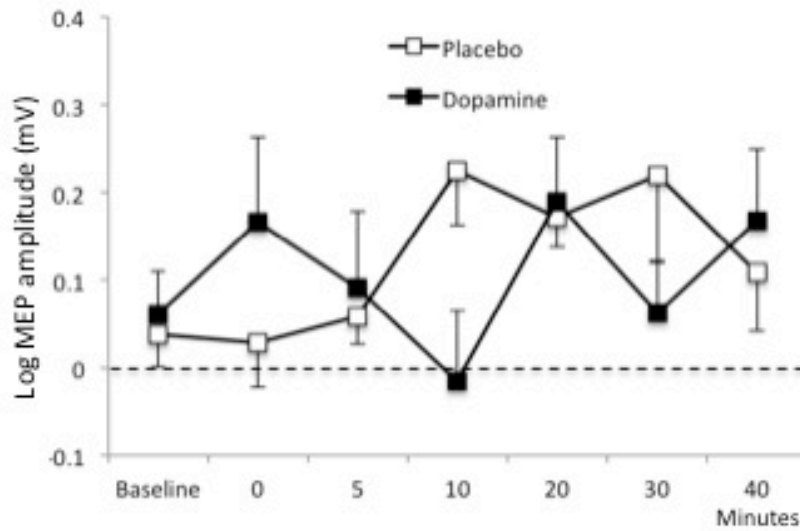


Figure 5.1.6. Effect of levodopa

The time course of the MEP amplitudes after iTBS in the placebo (squares) and levodopa (filled triangles) arms of the study is shown. Errors bars represent standard error of mean. Data for 6 subjects are shown.

In contrast to the nicotine experiment, therefore, no effect on the time course of corticospinal excitability following iTBS was observed for either dextro-amphetamine or levodopa. On this basis control experiments omitting iTBS were not performed for these 2 medications.

5.1.4 Discussion

In the present study we reproduce earlier reports showing a transient increase in corticospinal excitability in response to iTBS, and further show that this effect is enhanced and prolonged in the presence of nicotine. A control experiment demonstrated no effect of nicotine alone on corticospinal excitability, implying that this effect was the

result of an interaction between iTBS and nicotine. Similar effects were not observed for dextro-amphetamine or levodopa at the doses studied here.

5.1.4.1 The administration of nicotine

Nicotine on its own had no effect on MEP amplitudes in the present study. Unchanged corticospinal tract excitability in the resting state is consistent with a previous report testing the effects of acute nicotine administration (Orth et al 2005), in which there was a small increase in amplitudes recorded during tonic contraction (rather than at rest as tested in the present study) but both active and resting motor thresholds were unchanged. Thresholds were also unchanged in a recent physiological study in chronic smokers (Lang et al 2008). The use of questionnaires revealed that the blinding of subjects to nicotine administration (versus placebo) was ineffective here. This is likely to relate to the mild nausea commonly reported when naïve subjects are exposed to nicotine, a side-effect for which it would be difficult to control without potential effects on physiological measures. While the awareness of having taken the active agent may in theory affect cortical responses subjects were unaware of the hypotheses being tested, making it unlikely that any such awareness would exert a systematic effect on excitability. This is thus perhaps less of a confound than would be the case in a behavioural study.

5.1.4.2 Changes in corticospinal excitability with iTBS and nicotine

The transient increase in MEP amplitudes following iTBS delivered to the motor cortex was first described in 2005 (Huang et al 2005) and has been widely reproduced (Di Lazzaro et al 2008; Huang et al 2007; Huang et al 2008). With nicotine this increase was

more marked, started later and was more prolonged. Excitability was still increased at 40 minutes, and it is unclear how long this would have persisted. The absence of an effect with nicotine alone suggests that the effect resulted from an iTBS-drug interaction. This result is the first to our knowledge that demonstrates a pharmacological enhancement of the effects of TBS in humans.

It is interesting to note that the effect of iTBS in the placebo arm (Fig 5.1.3) appeared to have a shorter duration in comparison to previous reports: MEP amplitudes were increased for only 5 minutes in the present study, compared to 15 minutes in the original description of iTBS (Huang et al 2005). This is unlikely to relate to session order, which was randomised and counter-balanced. While the reason for the shorter duration is not clear one might speculate that it relates to the commonly reported inter-subject and inter-session variability in the effects of TBS and other plasticity protocols (Maeda et al 2000). The outcome of such experiments has been shown to depend on factors including genotype (Cheeran et al 2008), circadian rhythm (Sale et al 2008) and pre-existing motor state (Huang et al 2008; Gentner et al 2008). It has been noted in 2 prior studies that facilitation of excitability following iTBS appears to occur in 2 phases, with a dip at around 7-10 minutes, and it was speculated that these phases might have differing underlying mechanisms (Huang et al 2005, 2008). The baseline facilitation observed here would coincide with the first of these phases and it is possible that the second phase was absent in the group of subjects studied. It is important to note, however, that such factors would apply both to the placebo and to the nicotine arms and so should not affect the internal comparison made in the present study.

One interesting feature of our results is that although facilitation was enhanced by nicotine, its onset was delayed. Huang et al proposed concurrent inhibitory and facilitatory effects of theta burst stimulation, accumulating at different rates, in an attempt to explain the divergent effects of the intermittent and continuous paradigms on excitability (Huang et al 2005, 2011). In later experiments the same group also observed a dip in facilitation at around 7-10 mins in the intermittent form used here (Huang et al 2007), suggesting either a delayed inhibition at that stage or 2 separate phases of facilitation with different mechanisms. According to such a scheme the present results might thus be explained either by a slower accumulation of facilitation or alternatively by the enhancement of a second facilitatory phase with suppression of the first. A similar delayed onset of facilitation was observed with lorazepam in the effects of transcranial direct current stimulation (tDCS) without a concurrent change in intracortical inhibition (Nitsche et al 2004a). The authors proposed that the GABA-ergic influences of remote cortical and subcortical regions may explain such an effect: as measurement of intracortical inhibition was not part of the current protocol we can only speculate as to the role of GABA-ergic mechanisms here.

5.1.4.3 What is the mechanism of the observed interaction with nicotine?

There is some evidence that the increase in corticospinal excitability seen following iTBS occurs at least in part by induction of LTP (Huang et al 2007; Teo et al 2007). Nicotinic modulation of this synaptic strengthening would thus seem to represent a reasonable candidate mechanism for the interaction observed in the present study. However, in the

present study the facilitation of excitability induced by iTBS alone lasted only 5-10 minutes, and this shorter duration of effect must raise the possibility that mechanisms other than LTP are playing a role. Here we discuss how a nicotinic modulation of LTP might occur but we also consider other potential mechanisms for the observed facilitation, such as a reduction in GABA-ergic inhibition within the motor cortex or a direct cholinergic modulation of non-LTP-mediated synaptic plasticity.

There is evidence from animal models that nicotinic modulation of LTP can occur. Two subtypes of nicotinic acetylcholine receptors (nAChR) have been shown to be involved in LTP. The $\alpha 4 \beta 2$ nAChR which resides on the post-synaptic membrane enhances NMDA-dependent LTP by acting as a calcium channel (Matsuyama & Matsumoto 2003; Nakauchi et al 2008), while the presynaptic $\alpha 7$ nAChR subtype induces non-NMDA-dependent LTP by enhancing presynaptic neurotransmitter release (Matsuyama et al 2000; Yamazaki et al 2005; Welsby et al 2006; Lagostena et al 2008). The effect of nicotine on intermittent TBS could be mediated by either of these mechanisms. The interneuron-specific and lamina-specific distribution of nicotinic acetylcholine receptors suggests a complex role played by these receptors in modulating cortical plasticity.

Specific nicotinic modulation of LTP was demonstrated at the Schaffer collateral synapse onto CA1 pyramidal neurons in the hippocampus (Ge & Dani 2005). In the human motor cortex, autoradiographic studies suggest that nAChRs reside mainly in layers III and V, consistent with the site of LTP as demonstrated in rat motor cortex (Sihver et al 1998; Rioult-Pedotti et al 2000). When studied in the hippocampus, the acute administration of nicotine had a complex action on the 2 receptor subtypes whose net effect was to reduce

the GABAergic inhibition of pyramidal neurons (Alkondon et al 2000). A reduction in GABA-ergic inhibition as assessed by paired pulse TMS was not observed following nicotine administration in humans (Orth et al 2005). However, this measure is relatively robust and while it certainly depends on an action at GABA_A receptors (Ziemann et al 1996a) it is conceivable that it would not be significantly disturbed by a modest reduction in GABA-ergic activity. GABA-ergic modulation of plasticity induction at glutamatergic synapses has been documented both in the amygdale (Pan et al 2009) and the hippocampus (Matsuyama et al 2008), raising the possibility that such a mechanism may contribute to enhanced plasticity. Although we can only speculate as to the mechanism of the effect reported in the current study it seems feasible that acute nicotine administration could enhance the effect of iTBS by either or both of a reduction in GABAergic inhibition and actions at the glutamate synapse itself.

Cholinergic modulation has also been demonstrated in other models of motor cortical plasticity. Kuo and colleagues (2007) showed that an acetylcholinesterase inhibitor, rivastigmine, attenuated the effects of tDCS but enhanced the effects of paired-associative stimulation (PAS). While some of this effect may be mediated by the muscarinic AChR, inhibition of acetylcholinesterase would also enhance activation of nicotinic AChRs. Practice-dependent plasticity, a physiological phenomenon which is LTP-dependent, is also enhanced by acetylcholinesterase inhibition suggesting that this form of modulation may have physiological relevance (Meintzschel & Ziemann 2006).

5.1.4.4 Lack of effect of dextro-amphetamine or levodopa

Dextro-amphetamine enhances activity at both noradrenergic and dopaminergic receptors. Stimulation of either of these systems individually enhances use-dependent plasticity (Meintzschel & Ziemann 2006). Amphetamine itself enhances the MEP facilitation resulting from ischaemic nerve block, although suppressing the response to rTMS in this context (Ziemann et al 2002), and prolongs the effects of anodal tDCS on excitability (Nitsche et al 2004b). It has also been demonstrated to enhance practice-dependent plasticity in its own right (Tegenthoff et al 2004). Likewise, dopaminergic stimulation by an agonist enhances use-dependent plasticity, while producing a complex modulation of the response to direct current stimulation (Nitsche et al 2006). It is therefore perhaps surprising that no effect on the iTBS was observed with either medication. The iTBS-induced facilitation of MEP amplitudes in the placebo condition of the current study was more prolonged than previously described in the original reports of iTBS (Huang et al 2005), with facilitation above placebo persisting here at 20 minutes. One might speculate that this contributed to the lack of change with the 2 medications tested. Facilitation of use-dependent plasticity with levodopa has been described previously in the healthy elderly population (Floel et al 2008b): it may be that the young age of participants in the current study did not allow for additional plasticity in the presence of dopaminergic stimulation, which exerts an influence on plasticity paradigms in the form of an inverted U-shaped curve (Monte-Silva et al 2009). Without testing across a range of doses, and perhaps a range of ages, we do not believe that neuromodulation by these 2 medications can be ruled out by the current experiments.

5.1.4.5 Summary of conclusions

This study demonstrates that the effect of iTBS on corticospinal excitability is enhanced in the presence of nicotine: the lack of an effect with nicotine alone implies an interaction between the two. Possible explanations for this effect include a modulation of LTP but other synaptic mechanisms must also be considered. At a practical level, one might argue on this basis that smokers should be excluded from studies involving TBS. In view of the role played by synaptic plasticity in physiological processes such as motor learning, testing the behavioural correlate of this interaction is likely to represent an informative next step.

5.2 Effects of iTBS and neuromodulation on motor learning

5.2.1 Introduction

Intermittent Theta Burst Stimulation (iTBS) is a form of repetitive Transcranial Magnetic Stimulation (TMS) which transiently increases cortical excitability in healthy humans as measured by an enhanced amplitude of motor evoked potentials (MEPs) evoked from the primary motor cortex (Huang et al 2005). This increase depends upon activity at the NMDA glutamate receptor and is thought to involve synaptic strengthening in the form of Long Term Potentiation (LTP) (Huang et al 2005, 2007; Teo et al 2007). LTP plays a well-documented role in several forms of learning and there is evidence from synaptic studies in rats (Riout-Pedotti et al.2000) and physiological studies in humans (Muellbacher et al 2001; Butefisch et al 2000) that it occurs within the primary motor cortex when learning a new motor task. We therefore asked whether iTBS might interact

with the subsequent acquisition of a simple motor task, hypothesising that synaptic strengthening at NMDA receptors might enhance the outcome of this form of learning. Alternatively, if homeostatic metaplastic principles apply – where the effect of a plasticity intervention (i.e. facilitatory to inhibitory) can reverse according to the previous history of activity in the system (Ziemann et al 2004) – then iTBS would be counter-productive to learning. Additionally, recent studies of the effects of cholinergic agents on different types of plasticity have also suggested that these may be complicated by additional effects on levels of neural signal:noise ratio (Kuo et al 2007).

We demonstrated above that the LTP-like transient corticospinal excitability increase induced by iTBS is enhanced and prolonged when the subject is pre-medicated with nicotine. If such synaptic strengthening is important for acquiring a new motor task in humans then one might expect a similar synergism between iTBS and nicotine in their effects on the outcome of learning. However, the connection between the effect of nicotine on synaptic plasticity in one pathway and its effects on behaviour may be complex. Animal studies show that nicotine can produce both a pre- and post-synaptic enhancement of LTP (Fisher et al 1998; Mansvelder & McGehee 2000; Ji et al 2001; Ge & Dani 2005), but its effects on various forms of behavioural learning can be either facilitatory, absent or even inhibitory (Kenney & Gould 2008).

In order to address the question of whether iTBS can enhance the outcome of motor learning we tested the effects of iTBS (vs sham) and nicotine (vs placebo) on the subsequent acquisition of a simple well-characterised motor task. We performed a

randomised, double-blinded, placebo-controlled study in a 2 x 2 design with a group of 10 healthy subjects who were asked to maximise the peak acceleration of a ballistic thumb movement in a given direction across 6 blocks of practice. The nicotine arms were included in order to provide additional information regarding the role of synaptic strengthening via LTP in learning this task. We further performed a trial-by-trial analysis of the behavioural data, aiming to identify aspects of performance which may play a role in this form of learning.

Experiment 2 was conceived by the author but performed by Dr James Teo. The trial-by-trial behavioural analysis and subsequent computer modelling was performed by both the author and Dr Teo. Experiment 3 was conceived as an additional arm to this study and was performed by Dr Binith Cheeran.

5.2.2 Methods

5.2.2.1 Participants

Thirteen healthy right-handed subjects participated in this study. Ten subjects were included in Experiment 2 (mean age 29.5 ± 4.1 , mean \pm SD; 2 female) and six in Experiment 3 (mean age 29.3 ± 3.7 ; 2 female), with three subjects participating in both experiments. Subjects who smoked, or had smoked within the previous year, were excluded from the study. Subjects on any regular drugs (recreational or clinically-indicated) were excluded from the study. BDNF polymorphism status was not known for the majority of subjects.

5.2.2.2 Medication

Subjects were given either 2 mint-flavoured 2 mg nicotine lozenges (active medication) or 2 inactive mint lozenges (placebo). We chose lozenges in order to achieve rapid nicotine absorption, with maximum plasma levels reached in 60 minutes (Russell et al 1987; Tobacco Advisory Group 2000; Hukkanen et al 2005). In order to further disguise any taste associated with nicotine, all subjects were also given a strong-tasting menthol lozenge. Subjects were asked not to chew these lozenges, but to keep them in the mouth until fully dissolved.

5.2.2.3 Theta Burst Stimulation

Active motor thresholds were assessed and iTBS was performed (real and sham) as described in 5.1.2.3 above.

5.2.2.4 Experimental design

Experiment 2: motor learning

We used a randomised, placebo- and sham-controlled, blinded cross-over design with 4 sessions: (1) real iTBS-Nicotine; (2) sham iTBS-Nicotine; (3) real iTBS-placebo; and (4) sham iTBS-placebo. The order of pseudo-randomisation is shown in Table 5.2.1.

Subject	Session 1	Session 2	Session 3	Session 4
1	Sham-iTBS- Nicotine	Real-iTBS- Placebo	Real-iTBS- Nicotine	Sham iTBS - Placebo
2	Real-iTBS- Nicotine	Real-iTBS- Placebo	Sham-iTBS- Nicotine	Sham iTBS - Placebo
3	Sham-iTBS - Placebo	Real-iTBS- Placebo	Real-iTBS- Nicotine	Sham-iTBS- Nicotine
4	Sham-iTBS- Nicotine	Real-iTBS- Nicotine	Real-iTBS- Placebo	Sham iTBS - Placebo
5	Real-iTBS- Placebo	Real-iTBS- Nicotine	Sham-iTBS- Nicotine	Sham iTBS - Placebo
6	Sham-iTBS - Placebo	Sham-iTBS- Nicotine	Real-iTBS- Nicotine	Real-iTBS- Placebo
7	Real-iTBS- Nicotine	Sham-iTBS- Nicotine	Real-iTBS- Placebo	Sham iTBS - Placebo
8	Real-iTBS- Placebo	Sham-iTBS- Nicotine	Real-iTBS- Nicotine	Sham iTBS - Placebo
9	Sham-iTBS – Placebo	Real-iTBS- Nicotine	Sham-iTBS- Nicotine	Real-iTBS- Placebo
10	Sham-iTBS – Placebo	Real-iTBS- Placebo	Sham-iTBS- Nicotine	Real-iTBS- Nicotine

iTBS, intermittent theta burst stimulation.

Table 5.2.1. Order of pseudo-randomisation

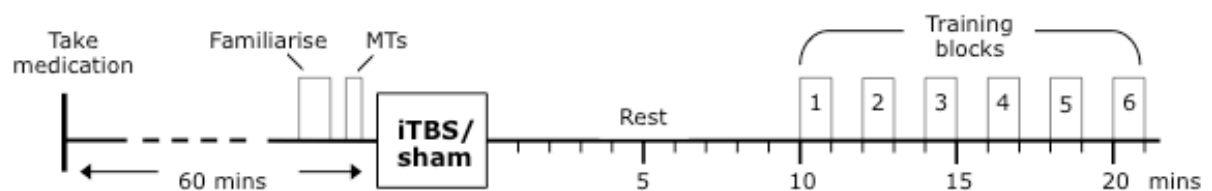


Figure 5.2.1 Outline of Experiment 2

The experimental outline is shown schematically in Figure 5.2.1 (above). 45 minutes after receiving the medication, subjects were familiarised with the task with 6 movements and the location of the hand area of the motor cortex was determined (see above). No

baseline measurement was performed as the very measurement of performance is likely to produce motor learning. Theta burst (or sham) was delivered 60 minutes after the medication was given, thus coinciding with the maximum plasma nicotine level (Hukkanen et al 2005). Subjects then rested for 10 minutes before starting to train in the task. We used a modified version of a well-characterised task in which behavioural improvement is associated with physiological changes in the primary motor cortex (Muellbacher et al 2001). The explicit goal of this motor learning task is to maximise acceleration in a particular vector for the thumb. This is analogous to motor skills involved in jumping or throwing sports that emphasise amplitude over directional accuracy (e.g. javelin or discus-throwing or long-distance jumping). Importantly, previous studies have shown that this form of motor learning is associated with physiological changes in the primary motor cortex, and interruption of the primary motor cortex after motor learning disrupts the motor memory (Muellbacher et al 2001, 2002).

The subject's left hand was positioned supine on a board with the wrist, metacarpophalangeal and distal interphalangeal joints fixed with Velcro straps. The thumb was left unsecured and could abduct and oppose freely. A piezoresistive monoaxial accelerometer (Model SA-105 vibrometer, Fribourg, Switzerland) was attached on the lateral aspect of the left thumb proximal phalanx with the maximal vector being thumb abduction. The accelerometer signal was sampled at 5000Hz and not filtered. The left non-dominant thumb was used in all conditions to minimise ceiling effects which may occur in the dominant hand. Subjects were asked to perform ballistic thumb abduction movements in time with a 0.5Hz audio metronome, with the explicit

aim of maximising the initial peak acceleration, using the computer monitor displaying the acceleration as a graph format in real time for visual feedback. This movement was chosen as it is more unnatural than a pinch and may therefore have greater scope for change in response to training. The monitor displayed the last three thumb abductions. The investigator motivated the subject by providing a target ~10% above the highest acceleration in the last three thumb abductions; subjects were made explicitly aware that the target provided was not an accuracy target but was there purely to motivate them to move faster on subsequent trials. Subjects were allowed ten movements for familiarisation before each experiment. Subjects were instructed to maintain the original thumb position by ensuring that the accelerometer signal returned to baseline (+0.05g) after each movement. Subjects performed 6 training blocks, each consisting of 30 training movements and lasting 1 minute. Training blocks were separated by rest periods of 1 minute. Session order was not counter-balanced (which would require 24 subjects) but the possibility of carry-over was reduced by leaving a minimum of 2 weeks (mean 31 days, range 14-55 days) between sessions.

Experiment 3: corticospinal variability

In order to assess the effect of iTBS on motor output variability in a manner independent of task performance, we measured vectors of thumb movements evoked by a single TMS pulse. This paradigm is similar to that employed by Classen et al (1998). A triaxial accelerometer (Entran Sensors & Electronics, Les Clayes-sous-bois, France) was placed on the left thumb proximal phalanx, allowing the derivation of a vector for each evoked thumb movement. We used a stereotactic neuro-navigation system (Brainsight, Rogue

Software, Montreal Quebec, Canada) to identify a location in the hand area of the primary motor cortex at which stimulation produces a TMS-evoked movement of a stable vector, defined by at least 8 out of 10 vectors lying within the same quadrant. After an initial baseline block (20 TMS-evoked movements), iTBS was then delivered to the same location of the motor cortex and a post-intervention block was recorded.

5.2.2.5 Data analysis and statistics

For experiment 2, accelerometer traces from individual trials were examined and those showing a premature or inadequate response (acceleration < 1g during the first 100ms of movement) were discarded. In remaining trials, the initial peak acceleration was measured as the difference between baseline and the first positive deflection peak and a mean value calculated for each block. Repeated-measures ANOVA was used to test for the effects of ‘time’ (or ‘block’), ‘drug’ and ‘stimulation’, and their interactions. Post-hoc paired t-tests uncorrected for multiple comparisons were used to examine differences with the control arm (ShamTBS-Placebo arm). The SPSS 12.0 package was used for performing statistical analysis.

A trial-by-trial analysis was performed retrospectively in order to produce a measure of performance variability for each session in Experiment 2. In order to allow us to measure variability with respect to a changing performance mean (as performance improves within the course of a given session), this measure was calculated iteratively in relation to a continuously changing derived ‘target’ acceleration. From trial 2 onwards, the outcome of each trial was tested for whether the acceleration exceeded the previous ‘target’ (set

for trial 2 as the value of the first trial): if so, then this target was increased by 50% of the difference between the new best outcome and the old target. The value of 50% was chosen in order that the changing target would not be excessively affected by isolated outliers. For each trial, the difference between the observed acceleration and the current target acceleration was calculated: the mean and standard deviation of this difference were determined for the session, and the coefficient of variability was calculated as (standard deviation / mean). This value thus reflects variability of the difference of the performance from the changing target value. Crucially, this measure is unaffected by the magnitude of performance or by overall change within a session. This approach of using a changing virtual ‘target’ value has the additional advantage that it reflects the subject’s immediate objective to maximise their performance compared to their memory of previous trials, and does not impose any preconceived learning rule on the analyses (which any simple curve-fit would do). Lastly, by expressing trial-to-trial variability in terms of the mean change (i.e. coefficient of variation) we should minimise any effects of signal dependent noise in motor output, since this also predicts that variability changes proportionally to the mean level of output (Hendricks & Robey 1936).

For experiment 3, the concentration parameter (κ) was derived from the TMS-evoked movement vectors using the circular statistics software Oriana (Oriana for Windows, Kovach Computing Services, Anglesey, Wales). κ is a measure of the directionality of the distribution (Fisher 1996) for which a value of 0 would represent no vector directionality (a distribution resembling a perfect circle), and thus maximal motor output variability.

We first performed the Rayleigh test on the movement vectors in order to verify that they were not circularly uniform. This confirmed that the κ is a valid measure of non-uniformity for this data set. We derived this measure at baseline, after iTBS and after no stimulation. κ is a non-linear parameter and was thus transformed with \log_{10} and a mean calculated for graphical representation. The non-parametric Wilcoxon paired signed ranks test was used to test for significant differences.

5.2.2.6 Modelling

We created a simple mathematical model of how the brain might approach improving performance in the thumb abduction task, with the aim of testing the effects of altering performance variability. This model was created and run in Microsoft Excel, with a macro programmed in Visual Basic (Microsoft Ltd, Seattle, USA). This model is similar to other models of reinforcement learning and stochastic motor learning in the literature (Sutton & Barto 1998; Schöllerhorn et al 2009). Details of this model's design are given in the Results section.

5.2.3 Results

5.2.3.1 Experiment 2

No subjects reported any side-effects. Subjects correctly identified the drug they had taken in a forced-choice questionnaire in 40% of nicotine sessions and 65% of placebo sessions: this is not significantly different from chance (Pearson Chi-Square test, $p=0.744$), suggesting that blinding was effective. Active motor thresholds and stimulation intensities are given in Table 5.2.2. After taking nicotine there was no significant

difference from placebo in motor thresholds (paired t-test, $P=0.590$), although in the stimulation intensity used for iTBS approached significant difference between nicotine and placebo arms ($P=0.054$).

Table 5.2.2. Experiment 2: physiological parameters

Condition	Active Motor Threshold (%MSO)	iTBS Stimulus Intensity (%MSO)
Real iTBS-Nicotine	41.4 ± 2.7	33.1 ± 2.2
Real iTBS-Placebo	45.4 ± 2.3	36.6 ± 1.8
Sham iTBS-Nicotine	45.4 ± 2.3	-
Sham iTBS-Placebo	43.0 ± 2.4	-

MSO, Mean stimulator output; iTBS, intermittent theta burst stimulation. Values given as Mean \pm SE.

Subjects' performance in the task is shown in Figure 5.2.2A: in each experimental condition, subjects successfully increased peak acceleration of the thumb abduction movement during the course of the 6 training blocks. The mean peak acceleration during Block 1 did not differ between the 4 conditions (2-way ANOVA with main factors 'Stimulation' and 'Drug': interaction, $F_{1,9}=0.6$, $P=0.456$; main effect of 'Stimulation', $F_{1,9}=0.9$, $P=0.371$; main effect of 'Drug', $F_{1,9}=0.3$, $P=0.597$), suggesting that subjects started training from a similar level of performance.

Although there was no statistical difference between groups in block 1, there is a suggestion that the iTBS-placebo group started at a higher acceleration than the other groups. Since each block consisted of 30 movements, it could be that this was caused by greater within-block learning. Therefore to obtain a better estimate of initial performance (after stimulation but before any learning) we also analysed only the first 5 trials across experimental arms to confirm similar baseline performance (Figure 5.6.2B). For the first 5 trials of Block 1, there was no significant effect on early performance (2-way ANOVA with main factors ‘Stimulation’ and ‘Drug’: interaction, $F_{1,9}=1.6$, $P=0.239$; main effect of ‘Stimulation’, $F_{1,9}=1.7$, $P=0.220$; main effect of ‘Drug’, $F_{1,9}=0.02$, $P=0.889$). If baseline was defined as the first 10 trials of Block 1, there was also no significant effect on early performance (2-way ANOVA with main factors ‘Stimulation’ and ‘Drug’: interaction, $F_{1,9}=0.5$, $P=0.508$; main effect of ‘Stimulation’, $F_{1,9}=2.3$, $P=0.164$; main effect of ‘Drug’, $F_{1,9}=0.4$, $P=0.520$).

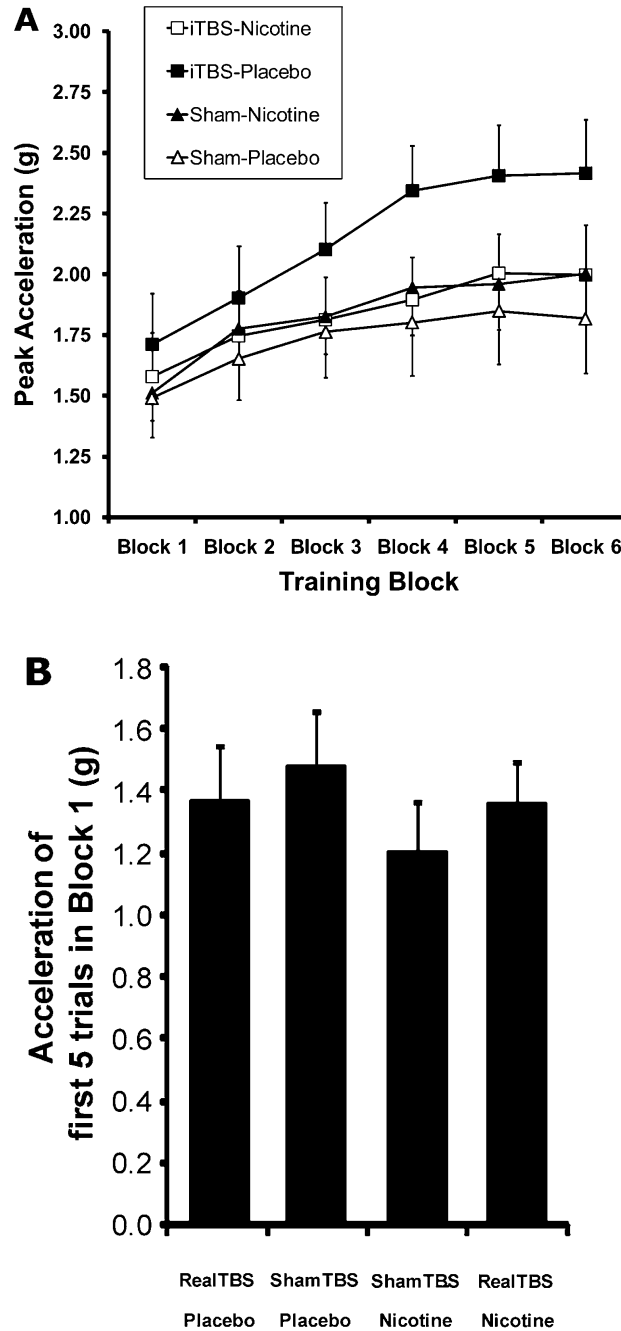


Figure 5.2.2 Outcome of training in Experiment 2

A. Group data showing the mean peak acceleration for each block in the 4 experimental arms. In each condition, subjects were successful in increasing the peak acceleration of thumb movements during the course of training. Although there were no significant differences in baseline performance, the change in

peak acceleration differed significantly across the 4 conditions (significant 3-way 'Stimulation' x 'Drug' x 'Block' interaction). Follow-up 'Stimulation' x 'Block' ANOVAs revealed a significant interaction in the placebo condition but not in the nicotine condition. iTBS thus enhanced learning in this task in the placebo condition, but this effect was absent in the presence of nicotine.

B. Baseline performance as measured as mean acceleration first 5 trials of Block 1. There was no significant effect or interaction of 'Stimulation' or 'Drug' on early performance.

The effect of training on peak acceleration was tested by comparing Block 1 with Block 6 in each condition: details of all ANOVAs are given in Table 5.2.3. A 3-way ANOVA with main factors 'Stimulation', 'Drug' and 'Block' revealed a significant interaction of these 3 factors ($F_{5,45} = 12.5$; $p=0.006$), suggesting that the increase in peak acceleration with training differed across the experimental conditions. The main effect of 'Block' was also significant ($P<0.001$), confirming that subjects successfully improved performance.

Table 5.2.3. ANOVAs for Experiment 2

ANOVA	Main factors	Degrees of freedom; error	Effects	F	P
3 way	Stimulation x Drug x Block:	5; 45	S x D x B	12.5	0.006
			S (main effect)	2.7	0.138
			D	0.6	0.445
			B	69.9	<0.001
2 way	Stimulation x Block: Placebo conditions	5;45	S x B	7.6	0.023
			S	3.7	0.086
			B	42.3	<0.001
	Nicotine conditions	5; 45	S x B	0.3	0.620
			S	0.1	0.780
			B	33.3	<0.001
2 way	Drug x Block: Sham conditions	5;45	D x B	2.0	0.191
			D	1.3	0.289
			B	16.3	0.003
	TBS conditions	5; 45	D x B	4.8	0.057
			D	2.1	0.178
			B	67.9	<0.001

S, Stimulation; D, Drug; B, Block; TBS, theta burst stimulation

Follow-up 2-way ANOVAs were performed and revealed a significant ‘Stimulation’ x ‘Block’ interaction in the placebo condition ($F_{5,45} = 7.6$; $P=0.023$) but not in the nicotine condition ($F_{5,45} = 0.3$; $P=0.620$). Thus iTBS significantly enhanced learning of the task with placebo, but this did not occur in the presence of nicotine. The main effect of ‘Stimulation’ was not significant (nicotine arms only, $F_{5,45} = 0.1$; $P=0.780$), but in the absence of nicotine, the effect of iTBS approached significance (placebo arms only, $F_{5,45} = 3.7$; $P=0.086$) which probably reflects the higher mean performance of all 6 blocks resulting from the increased performance in the latter 3 blocks.

Corresponding 2-way ANOVAs were performed testing the effect of nicotine on learning in the presence or absence of iTBS: the ‘Drug’ x ‘Block’ interaction approached significance in the TBS condition ($F_{5,45} = 4.8$; $P=0.057$) but not in the sham condition ($F_{5,45} = 2.0$, $P=0.191$). Finally, we found that there was no correlation between the initial performance of individual subjects and the extent of learning ($r^2 = 0.089$, $p = 0.583$).

Session order was pseudo-randomised across the group. However, in order to investigate the possibility that session number may have exerted a systematic influence on learning we performed a 2-way ANOVA with the main factors ‘Session number’ and ‘Block’. These factors did not interact (interaction, $F_{3,7}=3.2$, $P=0.092$; main effect of ‘Session number’, $F_{3,7}=1.0$, $P=0.445$; main effect of ‘Block’, $F_{1,9}=69.9$, $P<0.001$), suggesting that the observed effects of iTBS and nicotine were not a consequence of session order.

Taken together, these data indicate that improvement of peak acceleration in the course of training was enhanced by iTBS when subjects had taken the placebo, but that such enhancement was absent if subjects had taken nicotine. This blockade of the iTBS enhancement of motor learning by nicotine stands in contrast to the enhancement of the iTBS effect on corticospinal excitability by nicotine described in 5.1 above. This is difficult to explain solely in terms of enhanced LTP or homeostatic plasticity without invoking a complex rule relating the level of LTP and the ability to learn.

5.2.3.2 Model of learning in a ballistic motor task

Improvement in performance in the ballistic thumb abduction task depends on other factors in addition to learning / synaptic strengthening. The requirement to increase initial acceleration from trial to trial requires optimisation of motor commands to many motor units in a number of different muscles that operate around the metacarpophalangeal and wrist joints. Yet the only feedback available to subjects is a unidimensional indication of acceleration. Adjusting a multidimensional motor command on the basis of one-dimensional feedback is not a trivial problem, but one solution is to explore the task space by varying the motor commands from trial to trial. In this case, those combinations of motor output that improve performance are selected / remembered and then replaced when better combinations are found. To aid in understanding the factors involved in optimising performance, here we present a simple theoretical model of how a performance improvement may be achieved in such a situation; it is based on stochastic models of motor learning (Sutton & Barto 1998; Schöllhorn et al 2009).

The aim of our model was to allow us to investigate the effects of altering performance variability on the outcome, and thereby to determine whether it is feasible that such variability may have a positive effect on learning. This model was based on 2 assumptions: 1) that there is a maximum achievable peak acceleration; and 2) that the motor cortex has a fixed repertoire of possible outputs, each coding for different muscle groups, which can be discharged in parallel. Maximising the motor output would therefore involve determining the optimum weighting in which these motor outputs are to be discharged, presumably favouring task agonists over task antagonists. Thus the system must gradually solve a multi-dimensional problem using feedback given in one dimension, in the form of visual feedback from the previous trial. For the sake of our model we reduced the motor output repertoire to 2 dimensions, represented by 2 orthogonal axes x and y. The contribution of each axis to the observed motor output was defined by the same exponential function, such that for a given combination (x,y) the observed output is given by:

$$e^{-\left(\frac{x-75}{25}\right)^2} + e^{-\left(\frac{y-75}{25}\right)^2}$$

This function was chosen for the simple reason that it generates a motor output function with a single peak value, achieved at optimum values of x and y (see Figure 5.2.3A).

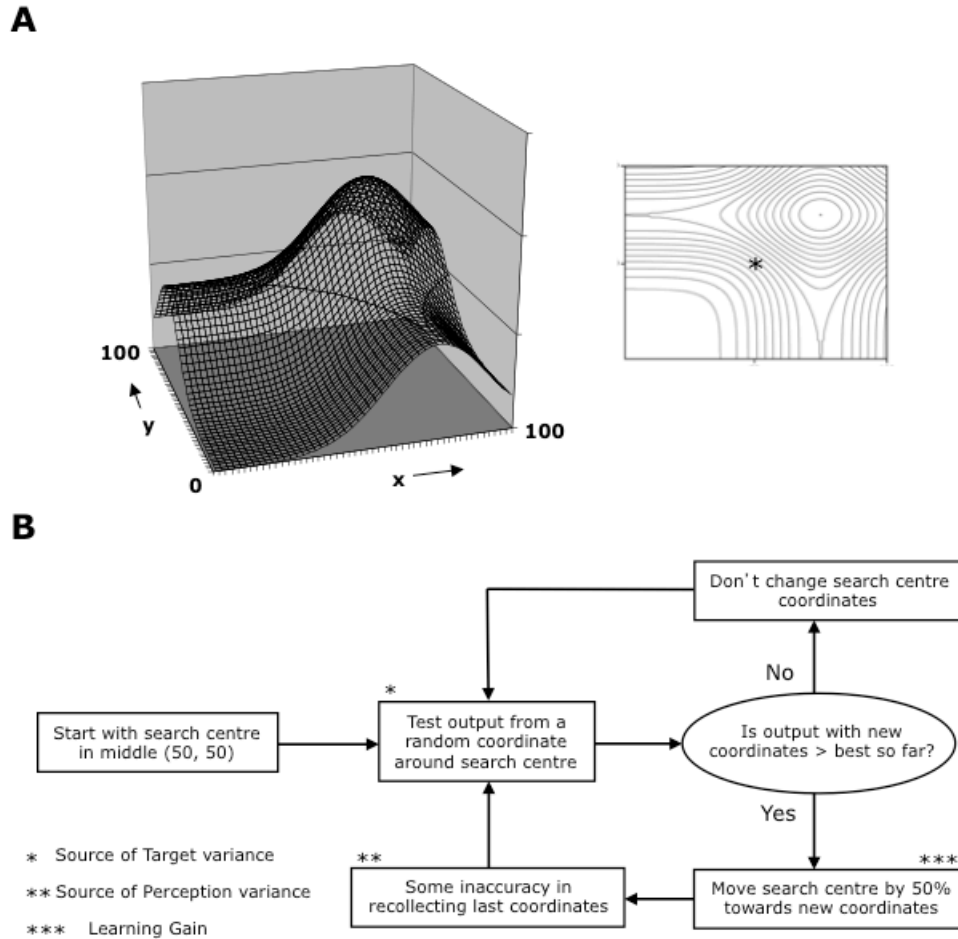


Figure 5.2.3 Model of learning strategy for task

The aim is to identify the optimum weighting of 2 simultaneously-discharged motor outputs (x and y), using the available performance feedback in the form of observed motor output.

A. The motor output function employed in the model. The observed motor output (vertical axis) was defined as the sum of the contributions of the 2 individual motor outputs (x and y), each of which obeyed an inverse exponential function such that there is a single peak which represents the maximum possible peak acceleration. In the contour view (right), the asterix represents the starting position.

B. The structure of the model is given in flow diagram form. Stochastic combinations of x and y are tried: if the resulting output of a given trial represents an improvement on prior performance then the search centre is moved in the perceived direction of the new coordinate. There are 2 distinct sources of variance, which remain fixed within a given run of the model: Output variance reflects the distribution of trials around the search centre, while Perception variance reflects error in the correct recollection of the previous trial coordinates. The proportion by which the search centre is moved towards the perceived previous coordinates in the event of a successful trial is termed the Learning Gain.

In our model the following iterative algorithm was applied:

- 1) Test the motor output at a stochastically-generated test point centred around the current search-centre coordinates (x,y) . The distance of this test point from the current search-centre coordinates obeys a normal probability density function, with a variance that remains fixed (the Output variance, OutputVar).
- 2) If the resulting output is an improvement on the best output so far, then the search-centre coordinates are updated – see step 3. Otherwise these coordinates remain unchanged and the model returns to step 1 (next iteration).
- 3) The search-centre coordinates are moved by a fixed proportional distance along a line joining the current search-centre with the system's perception of the most recent test coordinates. This perception of the test coordinates is not identical to the actual coordinates just used, in order to reflect a degree of error in both recalling the motor output just generated and in interpreting the afferent feedback from the resulting

movement. The distance of the perceived test coordinates from the real test coordinates also obeys a normal probability density function with a fixed variance (the Perception variance, *PerceptVar*). With the new performance coordinates, the model returns to step 1 (next iteration).

We term the extent by which the search-centre coordinates are adjusted (initially set at 50%) the Learning gain (*LearnG*), with a higher value denoting a greater degree of motor output change in response to given performance feedback. The Learning gain thus reflects the capacity for plastic change in this model. It may be noted that the model is simply an iterative implementation of a fixed set of rules – it does not include the capacity to discover underlying rules that find a solution more efficiently.

This model is represented in flow diagram form in Figure 5.2.3B (above). The initial test coordinates were always (50, 50), and 100 iterations were performed in the course of each run. The effects of varying either the *OutputVar* or the *PerceptVar* were examined by running the model 20 times at each set of values across a range and recording the resulting outputs. For each run of the model, the final output achieved was recorded as the mean of the last 10 trials (out of 100).

The respective effects of changing the *OutputVar* (with *PerceptVar* set at 14 and *LearnG* set at 0.1), the *PerceptVar* (with *OutputVar* set at 7 and *LearnG* set at 0.1) and the *LearnG* (with *OutputVar* set at 7 and *PerceptVar* set at 14) are shown in Figure 5.2.4.

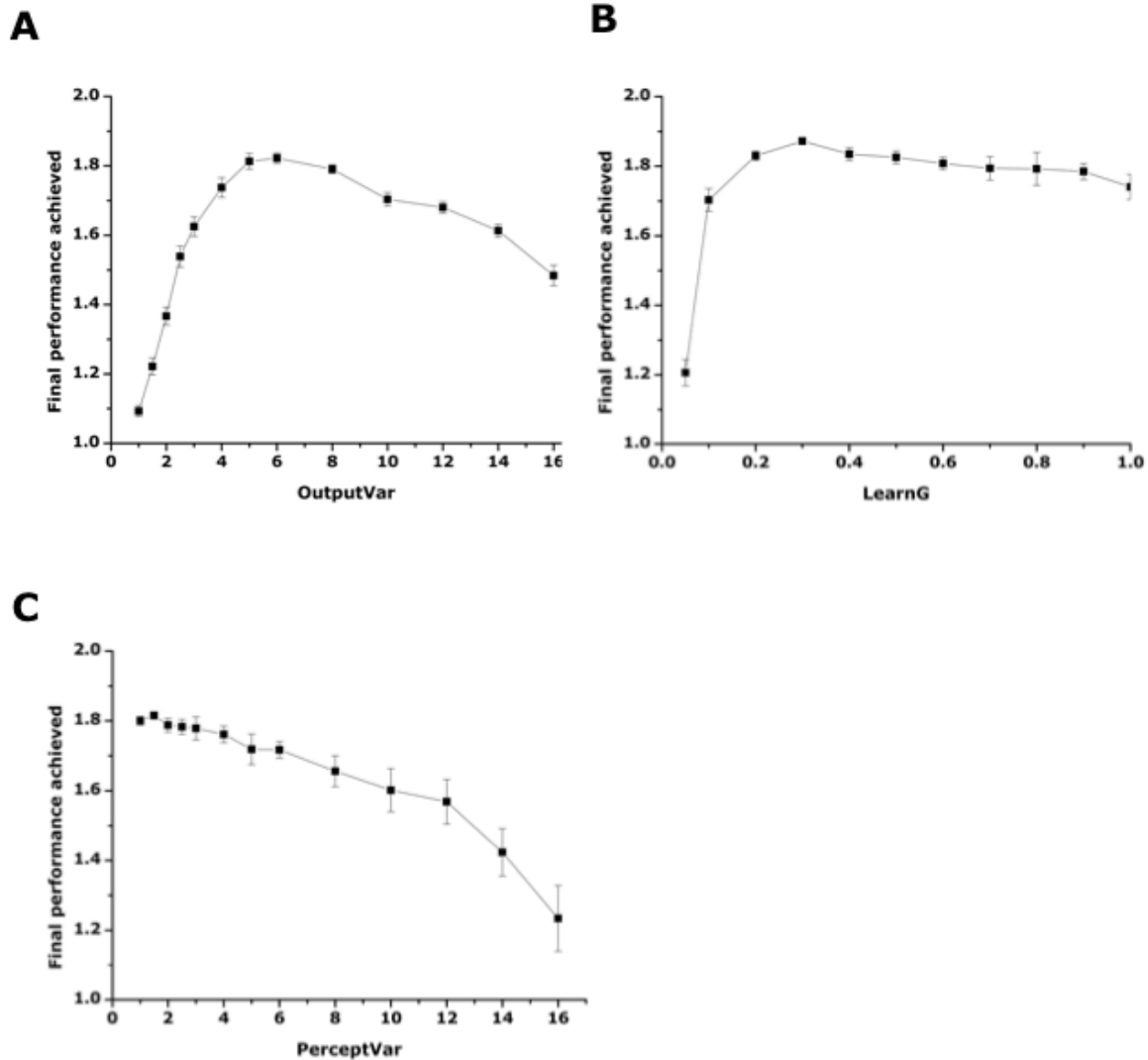


Figure 5.2.4. The effects of 2 forms of variance and learning gain on performance in the learning model

The model was run 20 times with each variance setting, and the final performance recorded as the mean of the last 10 trials (out of 100).

A. Altering output variability resulted in an inverted U-shaped curve, with an optimum value of this parameter. At the lower end of this curve increasing variability resulted in an improved outcome, with performance dropping off at higher values. We suggest that an increase in such ‘helpful variability’ may account for the improved learning observed following iTBS in Experiment 1.

- B.** Learning gain is also associated with an inverse U-shaped curve, with a decline in final outcome at more extreme values.
- C.** Perception variability, in contrast to performance variability, was entirely detrimental to final performance in this model.

At each setting the final performance depended crucially on the variables tested. For OutputVar (Figure 5.2.4A), low values resulted in poor final performance, with little improvement across the 100 trials. Increasing OutputVar was initially beneficial, but beyond an optimal value further increases resulted in impaired performance. For LearnG (Figure 5.2.4B), increases resulted in a similar inverse-U-shaped curve with an optimum value beyond which further increases were detrimental to performance. For PerceptVar (Figure 5.2.4C), by contrast, there was no optimal value – increasing this form of variability resulted in a steady decline in performance. In order to address the possibility that the different curve shapes observed for OutputVar and PerceptVar were a result of the LearnG value chosen, these analyses were repeated across a range of LearnG values, demonstrating that this distinction applied at all 3 values tested (Supplementary Figure 5.1). This approach further demonstrated that the optimal value for OutputVar was higher at lower values of LearnG, suggesting that there is a greater beneficial effect of output variability when the intrinsic capacity for plastic change is low.

Within our simple model of a motor task, therefore, there will be an optimal amount of variability that allows the fastest possible exploration of all possible muscle combinations. On this basis, we examined the results from our ballistic motor task

(Experiment 2) using a trial-by-trial basis analysis to test how iTBS affected trial-by-trial variability.

5.2.3.3 Trial-by-trial analysis of performance in Experiment 2

We first tested the possibility that in the course of 6 blocks subjects were subconsciously perceiving and exploiting an underlying cognitive rule allowing them to improve their performance more effectively: if this were the case then perhaps iTBS might facilitate such a process, thus improving learning outcome, and would invalidate the model described. Within a block, the probability that an individual trial would be better than the previous trial was calculated with a simple formula:

$$P = t(x) / T$$

where P is the probability of an individual trial being better than the previous trial; $t(x)$ is the number of trials where performance in x^{th} trial is greater than $(x-1)^{\text{th}}$ trial; and T is the total number of trials in the block. If iTBS were improving motor learning via an effect on such cognitive rule acquisition then the iTBS sessions should have a higher P than sessions with a sham intervention.

Values for P in each session type were stable over the course of the 6 blocks (shown in Supplementary Figure 5.2). In a 3-way ANOVA with the within-subject factors ‘Stimulation’, ‘Drug’ and ‘Block’ the main effect of ‘Block’ was not significant ($F_{5,45}=1.673$, $p=0.161$), suggesting that no cognitive rule acquisition was contributing to performance improvement in this task. Furthermore, there was no interaction of the 3 within-subject factors nor any effect of iTBS within the Placebo arms ($F_{5,45}=0.903$,

$p=0.488$). The lack of an interaction thus makes it unlikely that such a process might explain the improved learning with iTBS.

Trial-by-trial variability was measured, using a method that controls for the gradual improvement that occurs with training (see 5.2.2.5 above): a representative example of how this measure was derived is shown in Figure 5.2.5A, and group data is presented in Figure 5.2.5B.

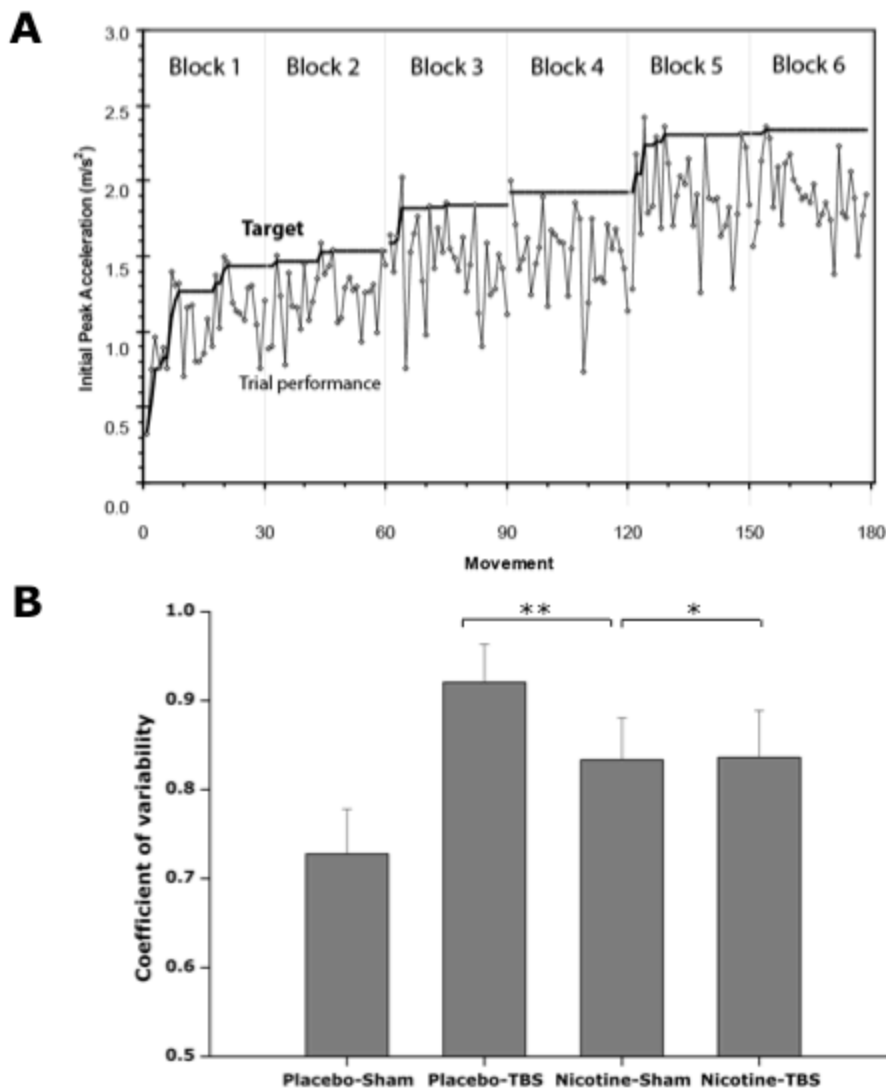


Figure 5.2.5. Performance variability during Experiment 2

A. Example of a trial-by-trial measure of peak acceleration (diamonds, plain line) with a derived ‘moving target’ (bold line) which increases every time it is exceeded by a new trial. Coefficient of variability is thus derived from the variability of the difference between the trial acceleration and the ‘target’.

B. The mean coefficient of variability is given for each session type. The effect of iTBS (vs sham stimulation) on variability was different in the nicotine and placebo sessions, as revealed by a significant interaction between the factors Stimulation and Drug (see text for ANOVA details). In the placebo sessions, performance variability was significantly greater after TBS than after sham stimulation (** $P < 0.001$), whereas TBS did not affect variability in the presence of nicotine ($P = 0.965$). Moreover, in the sessions with TBS performance variability was significantly reduced in the presence of nicotine ($P = 0.031$).

A 2-way ANOVA revealed a significant interaction between the factors ‘Stimulation’ and ‘Drug’ ($F_{1,9} = 7.637$, $P = 0.022$), with a significant main effect of Stimulation ($F_{1,9} = 9.265$, $P = 0.014$) but not of Drug ($F_{1,9} = 0.150$, $P = 0.707$). This interaction was explained by significantly greater performance variability after TBS than after sham stimulation in the placebo sessions (Paired t-test, $P < 0.001$) but not in the nicotine sessions ($P = 0.965$). A comparison of the TBS sessions (nicotine vs placebo) revealed that performance variability was significantly reduced in the presence of nicotine ($P = 0.031$). The results of this analysis suggest that iTBS had the effect of increasing performance variability when the subject had taken placebo, but that this effect did not occur if they had taken nicotine: this was the same pattern observed in the effect of TBS on learning.

We further tested whether performance variability and learning were related in these experiments. When these variables were plotted for all 40 sessions regardless of session type (Figure 5.2.6) there was a positive correlation between performance variability and total learning, defined as (Block 6 mean performance – Block 1 mean performance) ($r^2=0.301$, $P<0.001$). This supports the idea that the beneficial effects of iTBS on learning observed here may relate to a modulation of performance variability.

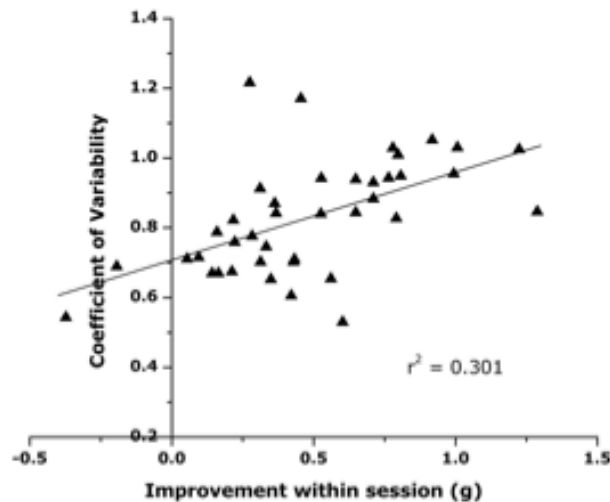


Figure 5.2.6. Greater performance variability was associated with a more successful learning outcome

The learning sessions were combined (regardless of session type) in order to examine the relationship between the coefficient of variability and the extent of the total improvement achieved across the 6 training blocks. There was a strong correlation between these variables, such that greater performance variability was associated with greater learning ($r^2 = 0.301$, $P<0.001$).

In order to provide reassurance that CoeffVar does in fact provide a measure of performance variability we tested it by analysing data from the simulation model. This

demonstrated a clear positive slope for CoeffVar with increasing values of OutputVar, suggesting that the CoeffVar does indeed reflect variability in this model of a learning task (Supplementary Figure 5.3).

Another possibility for the improving performance may be due to changing kinematic properties or changing contractility of unrecorded muscles. This study cannot completely exclude this possibility, although if these properties were responsible one would not expect the changes to continue to improve with repeated practice over 6 blocks.

Alternatively, ‘voluntary effort’ may be responsible for the performance changes: this possibility is explored in Experiment 3.

5.2.3.4 Experiment 3: Effect of iTBS on variability of evoked thumb movements

It should be noted that separating out the trial-to-trial variability in performance during experiment 2 into a random element and an underlying learning curve is dependent on how the trend in learning is defined. Our model explicitly requires that mean performance should tend to increase; fitting other models in which this is not the case may lead to different estimates of random variation. We therefore searched for other evidence that iTBS might affect the variability of motor output

In experiment 3 we tested the effect of iTBS on the directional variability of a TMS-evoked thumb movement: this measure is obtained at rest and is independent of movement magnitude. It is also independent of any variation in volitional motor commands, and therefore is an unbiased estimate of trial-to-trial variation in the

distribution of excitability in the corticospinal output to different muscles acting on the thumb. This is shown in Figure 5.2.7.

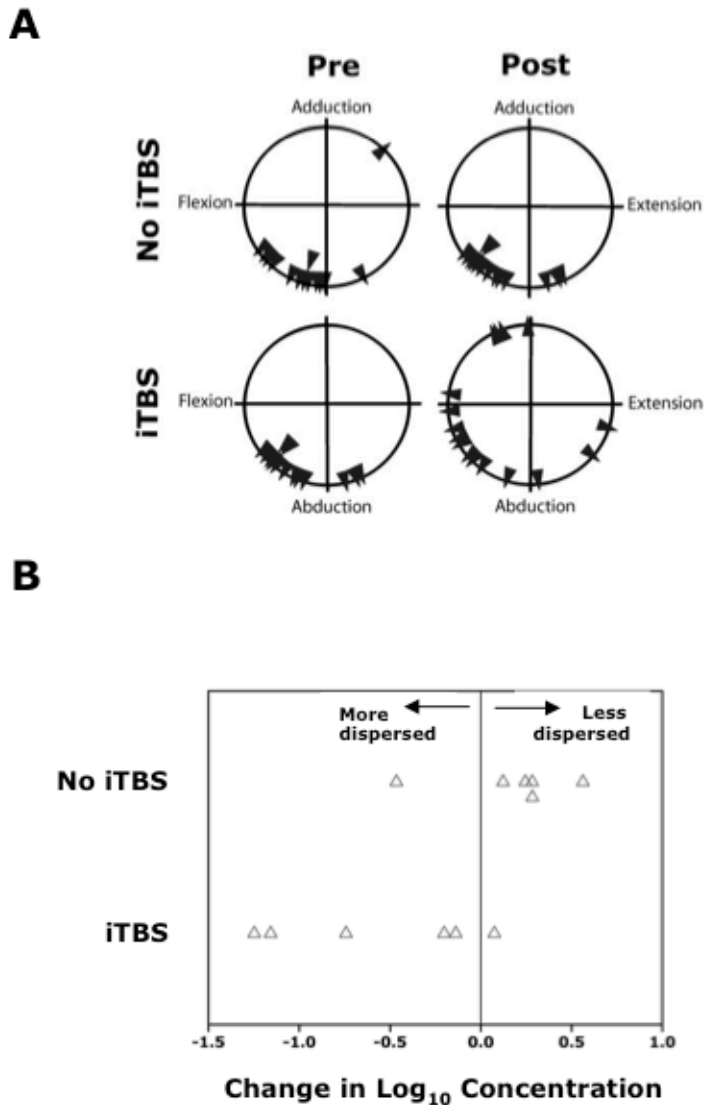


Figure 5.2.7. Experiment 3: the effect of iTBS on the variability of TMS-evoked thumb movements

A. Directional vectors for 20 consecutive TMS-evoked thumb movements are shown for a single representative subject. At the start of each session, and after no intervention, the direction of thumb movement was stable. Following iTBS the direction of evoked

movements became more variable, such that the concentration parameter (κ) was reduced.

B. The change in concentration parameter κ following either iTBS or no intervention is shown for each subject. A negative change in κ denotes an increase in the variability of TMS-evoked movement vectors.

Baseline values did not differ between the 2 session types (Wilcoxon paired signed rank test $P=0.249$). κ was significantly reduced following iTBS (0.046) but not after no intervention (0.249), indicating that iTBS increased the variability of TMS-evoked thumb movements.

Figure 5.2.7A shows data from one representative subject, in which the direction of TMS-evoked movement was considerably dispersed following iTBS but remained stable after no intervention. In Figure 5.2.7B the change in statistical concentration (κ) of TMS-evoked movement vectors following either iTBS or no intervention is shown for each subject. A lower value for κ denotes a greater degree of variability, so that a negative change in this parameter indicates an increase in movement dispersion. The baseline value for κ did not differ between the 2 session types (Wilcoxon paired signed rank test $P=0.249$). In the sessions without stimulation κ was not significantly changed at the post-intervention time point (Pre 0.677 ± 0.159 (mean \pm SE); Post 0.849 ± 0.219 ; $P=0.249$). Following iTBS, by contrast, there was a significant reduction in κ (Pre 0.924 ± 0.183 ; Post 0.355 ± 0.183 ; $P=0.046$): iTBS was therefore associated here with an increase in directional variability of the TMS-evoked motor output.

5.2.4 Discussion

The present task required subjects to maximise the initial acceleration of a thumb abduction movement. Thus they had to optimise the motor output to a number of

different muscles operating at the joint on the basis of a single feedback variable. We constructed a simple model of learning in such a situation showing that performance improvement not only depends on remembering sets of successful motor commands, but also on variability of motor outputs to seek even better patterns of muscle activity. When we analysed trial-to-trial performance according to this model we found that iTBS improved the rate of learning and enhanced performance variability; in addition, both effects were suppressed when iTBS was given in the presence of nicotine. Experiment 3 provided support for the assumptions of the model by showing that iTBS also increased variability in the direction of thumb movements evoked by single pulse TMS of motor cortex, indicating that increased variability of movement can be attributed at least in part to the effect of iTBS on the corticospinal output population at the level of the primary motor cortex.

5.2.4.1 The effect of iTBS on task acquisition

iTBS improved the outcome of training in this task without significantly altering baseline performance, suggesting an effect on learning rather than on overall performance.

Performance after iTBS was still improving at the end of training, so it is not clear whether a plateau had been reached or if further improvements may have been expected with continued training. To our knowledge, this is the first report in healthy volunteers in which excitatory rTMS, rather than a paired associative protocol (e.g. Jung & Ziemann 2009), enhances subsequent motor learning. A recent study found no effect of 5 Hz rTMS on training in a finger abduction task (Agostino et al 2007; Agostino et al 2008).

However, this study involved training both before and after rTMS, with likely consequent

carry-over and online feedback of accelerometer readings was not provided which is central to performance improvement based on our model of this paradigm.

A recent study (Reis et al 2009) found a facilitatory effect of anodal TDCS on performance during motor skill acquisition ('online learning') as well as early consolidation ('offline learning'). The facilitatory effect of a non-invasive stimulation paradigm on 'online learning' has some superficial similarities to our study, although the different learning paradigms (force generation learning in our study compared to speed-accuracy trade-off learning) make it difficult to compare easily. Additionally, our study does not explore the effects of iTBS on 'offline learning'.

By what mechanism might iTBS have enhanced performance improvement in this study? Improvement in such a task, with no discernible rules by which to improve performance, must involve two processes. First, there must be a driver to change, such that performance on a trial differs from, and on average is better than, that of the previous trial. Second, any beneficial changes in output should be recognised and stabilised, perhaps by changes in synaptic connectivity. The latter of these processes would seem to be a reasonable target for modulation by iTBS. Motor learning is known to be accompanied by LTP within the primary motor cortex (Rioult-Pedotti et al 2000), presumably resulting in synaptic strengthening in selected pathways. iTBS is thought to act at a cortical level (Di Lazzaro et al 2008) and can promote changes in synaptic strength (Huang et al 2005, 2007) that are thought to involve an LTP-like effect (Huang

et al 2007; Teo et al 2007): perhaps enhanced learning following iTBS may occur via increased synaptic activity with up-regulation of LTP.

Jung & Ziemann (2009) recently investigated the interaction of motor learning with LTP-like and LTD-like effects produced by paired associative stimulation of median nerve and TMS of cortex. They found that both LTP-like and LTD-like effects improved learning when applied immediately before practice. In contrast, if there was an interval of 30 min between PAS and learning then a homeostatic interaction was seen: LTP-like PAS reduced the rate of learning whilst LTD-like PAS increased the rate of learning. They argued that if learning followed PAS without any interval, then homeostatic effects were obscured by other mechanisms. These could involve the transient blockade of LTD that has been described after induction of LTP (Peineau et al 2007), which would prevent any LTD-like depression of learning. In addition, they suggested that as long as the amount of LTP induced by PAS was not saturated then this could facilitate subsequent learning (Berger 1984; Jeffery & Morris 1993). In the present experiments, subjects rested for 10 min between application of iTBS and start of the learning task. This was because previous work had shown that it can take several minutes for the effects of iTBS to produce stable effects on cortical excitability, and we wanted to maximise potential interactions with learning. Given the results of Jung & Ziemann (2009), it may be that if subjects had started to learn the task immediately after iTBS, then quite different effects would have been observed. Finally it is worth noting that different types of plasticity-inducing protocols in humans may interact with learning in different ways. For example, Kuo et al (2008b) found virtually no effect of prior conditioning with either anodal or

cathodal TDCS (with or without pre-treatment with the partial NMDA receptor-agonist d-cycloserine) on sequence learning in a serial reaction time task.

5.2.4.2 The effect of the iTBS-Nicotine interaction on learning

The lack of effect of nicotine on training in the placebo arm and the counter-productive effects when combined with iTBS were arguably surprising results. Cholinesterase inhibitors can enhance the outcomes of both paired associative stimulation and use-dependent plasticity experiments in humans, protocols thought to depend on LTP-like processes within M1 (Meintzschel & Ziemann 2006; Kuo et al 2007). One may therefore have expected nicotine to enhance task acquisition in the present study, but this was not the case.

In the presence of nicotine the positive effect of iTBS on subsequent task acquisition was blocked, with subjects improving at the placebo rate. One way to interpret this result would be to suggest, as above, that learning can be improved by interaction with non-saturated LTP induced by iTBS, but that addition of nicotine increases LTP to the point that it is saturated to further behavioural learning. Indeed, we demonstrated in Experiment 1 above (5.1.3) that addition of nicotine to iTBS increases cortical excitability (and perhaps LTP) more than iTBS alone. Thus we could propose that there is an inverted ‘U’ shaped interaction between the amount of LTP and facilitation of learning.

However, such arguments are necessarily speculative and link changes in measures of cortical excitability to complex processes of synaptic LTP and LTD in too simplistic a manner. This raises the question as to whether factors other than changes in synaptic efficacy may have been responsible for the positive effect of iTBS when given without nicotine. As discussed above, improvement in this task must also involve trial-to-trial variations in performance in order to achieve performance change: we therefore investigated the effects of our 2 interventions on performance variability.

5.2.4.3 The effects of the 2 interventions on motor output variability

Performance variability in Experiment 2 was modulated in a similar pattern to that observed for learning outcome: iTBS increased variability but this effect was blocked by nicotine. Moreover, performance variability in a given session was correlated with the behavioural gain observed. This result raises the possibility that iTBS may have enhanced the outcome of training by increasing the trial-by-trial variability of movements, thereby driving performance change. In drawing this conclusion it is important to be confident that an apparent increase in variability does not simply represent a scaling effect resulting from larger movements (Jones et al 2002; Hamilton et al 2004). This is unlikely to be the case, as the variability measure was centred at a baseline that increased with improving performance and the resulting statistic was normalised to the magnitude of movements in the given session (see 5.2.2.5 above). This normalisation against baseline would control for signal-dependent noise (Hamilton et al 2004).

In Experiment 3, iTBS also increased the directional variability of evoked movements. This experiment involves no voluntary contribution from the subject, who remains at rest throughout, suggesting an effect on variability that is independent of fluctuations in voluntary drive. A change in motor output variability in this context is likely to relate to processes occurring within the primary motor cortex itself – this is perhaps not surprising as this was the site at which iTBS was delivered. We conclude that iTBS increases motor cortex output variability, probably through increasing synaptic efficacy non-specifically in an area of motor cortex and that this is somehow beneficial to performance improvement.

Nicotine blocked the iTBS-related increase in performance variability, but did not alter variability on its own. A recent study in humans has suggested that cholinergic stimulation may increase the signal-to-noise ratio in the motor cortex (Kuo et al 2007). Similarly, nicotine increases the gain in thalamic inputs to the visual cortex (Disney et al 2007). In this context, the present results may be explained in these terms if the focusing effect of nicotine were to reduce variability within the motor cortex, with a consequent negative effect on learning.

5.2.4.4. Motor output variability and learning

Variability is an inherent feature of motor performance, arising from noise in both motor planning (Churchland et al 2006), execution (van Beers et al 2004) and motor learning (Huang & Shadmehr 2009; Braun et al 2009). The idea that increasing variability may improve learning may initially seem counter-intuitive but has been described (Patton et al

2006), and a formal model where motor learning can occur in the presence of stochastic perturbations (or can even be enhanced by variability) has recently been proposed (Schöllhorn et al 2009). In our model of the task, we have assumed that the subject is unable to formulate a set of cognitive rules to aid improvement from the information available about the current trial. This contrasts, for example, with sequence learning in which knowledge of the sequence itself predicts the optimal output on each trial.

In this model, the drivers to performance change were stochastic variations in motor output (OutputVar) and in the memory of the previous trial's output (PerceptVar). The latter may be particularly important in the absence of cognitive rules to guide improvement, as the subject must be able to recall the features of a successful trial in order to attempt to reproduce it. The effects of altering the two forms of variability in our model were fundamentally different. While variability in accurate recollection of the previous trial (PerceptVar) was entirely detrimental effect to final performance, the same was not true for variability in search-centre coordinates (OutputVar) where an inverted U-shaped curve was observed. Increasing OutputVar allows the model to try a wider range of combinations, allowing the system to 'escape' a performance plateau and continue improving. This is akin to a selection process where a degree of diversity allows a gradual evolutionary change to occur.

On the other hand, excessive OutputVar adversely affects the reproducibility of good movements so that learning suffers. The LearnG determines the extent of output adjustment made in response to an improvement and so reflects the degree of plasticity

available. A relatively small amount is required for optimal learning, beyond which improvement declines. Impaired performance at higher values of LearnG was explained here by greater system variability, suggesting a complex interaction between plasticity and variability. Thus, a highly variable system would benefit from less plasticity (due to the risk of learning an error) while a less variable system would benefit from greater plasticity.

It is also worthwhile to note that although the motor output variability is stochastic and random, intrinsic to our model is the comparator between current performance and the motor memory of best performance; this would tolerate variability resulting in better performances but would reject variability resulting in poorer performance. This comparator would have its own intrinsic variability which would itself be detrimental to performance. In our model, the term PerceptVar can be viewed as combining in a single measure the variability of both the comparator and the true perceptual variance. It may be further noted that performance in our subjects appeared not to have reached a plateau by the end of training, suggesting that they remain on the rising slope of the outcome graph in Fig 5.3 and that with given variability parameters further gains may have been achieved with longer training.

Finally, we do not exclude the possibility that iTBS may also have induced changes in LearnG, and that these could contribute to changes in performance. However, increasing LearnG (just like OutputVar) does not always correlate with better performance. Indeed,

as indicated by the model, the net behavioural effect of an intervention depends on a combination of its influence on LearnG and OutputVar.

These results are obtained from a simple model that shows how the motor system might operate in the absence of cognitive-rule-based optimisations and in the presence of stochastic variability. Our model of motor learning combines elements from differential motor learning (Frank et al 2008) although the presence of a clear driving imperative to increase performance has some similarities with schema theories of learning (Schmidt et al 1975). Nonetheless, even in such a simple model, there appears to be an interaction between stochastic variability and plasticity with respect to net performance gain, in line with our experimental data.

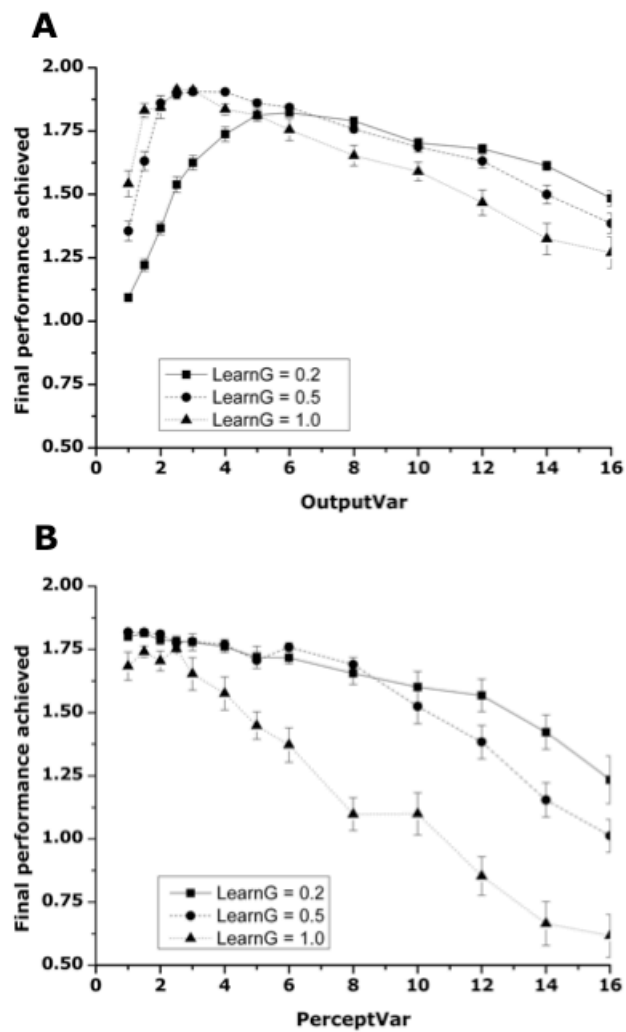
5.2.4.5 Limitations

There are some limitations to this study: as the experimental sessions were pseudo-randomised and not completely counter-balanced, there may be an effect of order. Certainly there may be a trend towards a significant interaction ($F_{3,7} = 3.2$, $p=0.092$) in a 3-way ANOVA (within-subject factors ‘Stimulation’, ‘Drug’ and ‘Session number’). One way to avoid the carry-over effect would be to design future studies to compare separate groups of subjects in each arm rather than a similar group of subjects across several arms; this will be logistically more challenging as one has to ensure that groups are appropriately matched in terms of handedness, age, skill and genotype.

Another limitation is this study did not investigate continuous TBS. It would be interesting to test if the effect also holds true for an inhibitory rTMS paradigm, which may have similar or different effects depending on the net effect on signal-to-noise ratio. It would be difficult to predict the net effect on signal-to-noise ratio of a particular intervention as the effect on the different parameters (OutputVar, PerceptVar and LearnG) may be different for different interventions, making the effect of an intervention unpredictable. Certainly, other studies have shown an effect of tDCS on the signal-to-noise ratio in a visuomotor task (Antal et al 2004).

5.2.4.6 Conclusions

In this series of experiments we have shown that this form of rTMS is capable of enhancing the subsequent acquisition of a simple ballistic motor learning task. Analysis of the behavioural data suggests that there may be a beneficial role for motor variability in this task and our simple model confirms that this is feasible. However, it is likely that in other motor learning tasks increased variability is disadvantageous, for example in tasks where the goal is precision or accuracy. This study does not discount a role for LTP in motor learning, but the dissociation between neurophysiological parameters and behavioural effects in this study (and other studies) emphasises the need for behavioural and computational aspects of motor learning to be appreciated if one hopes to understand or even modulate motor learning.

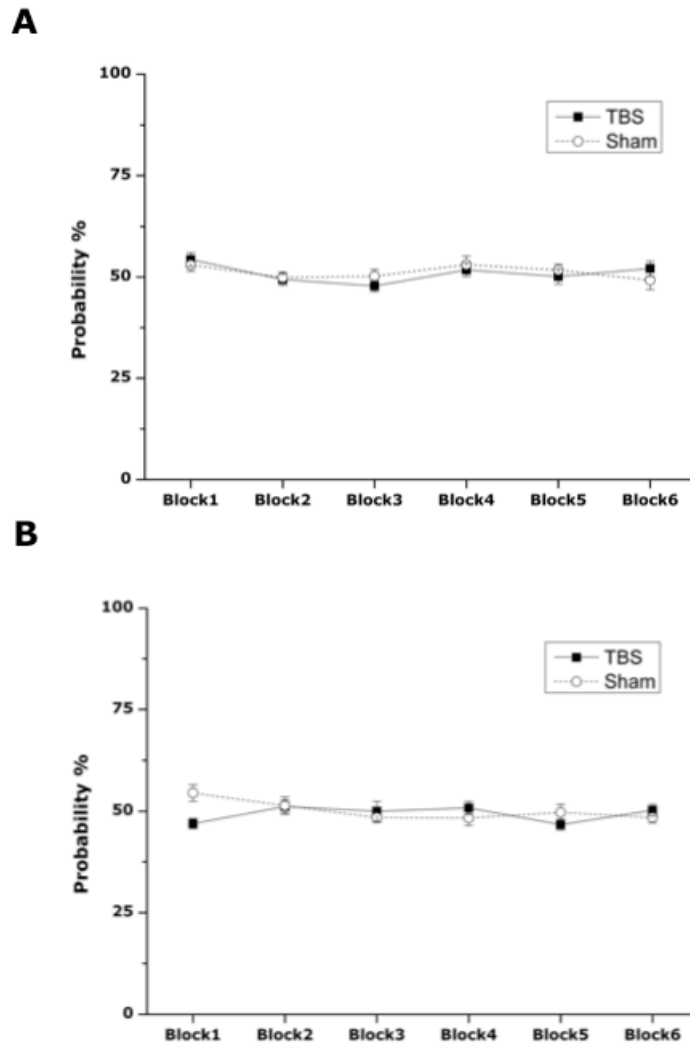


Supplementary Figure 5.1

The influence of Learning Gain on the relationship between variability and performance in the learning model

A. For output variability (OutputVar) the U-shaped curve shown in Figure 5.2.4 persists across the 3 values of LearnG tested: with increasing OutputVar learning initially increases and then drops off, such that there is an optimum value of OutputVar for each setting of LearnG. Interestingly, the curves for the low and high values of LearnG intersect. This suggests that in this model there is an interactive relationship between plasticity (LearnG) and output variability: when the capacity for plasticity is low then effective learning is favoured by greater output variability, while in the context of greater plasticity less variability is favourable.

B. For perception variability (PerceptVar) the curve shown in Figure 5.2.4 is similar across the 3 values of LearnG tested: increasing PerceptVar is consistently detrimental to learning outcome.

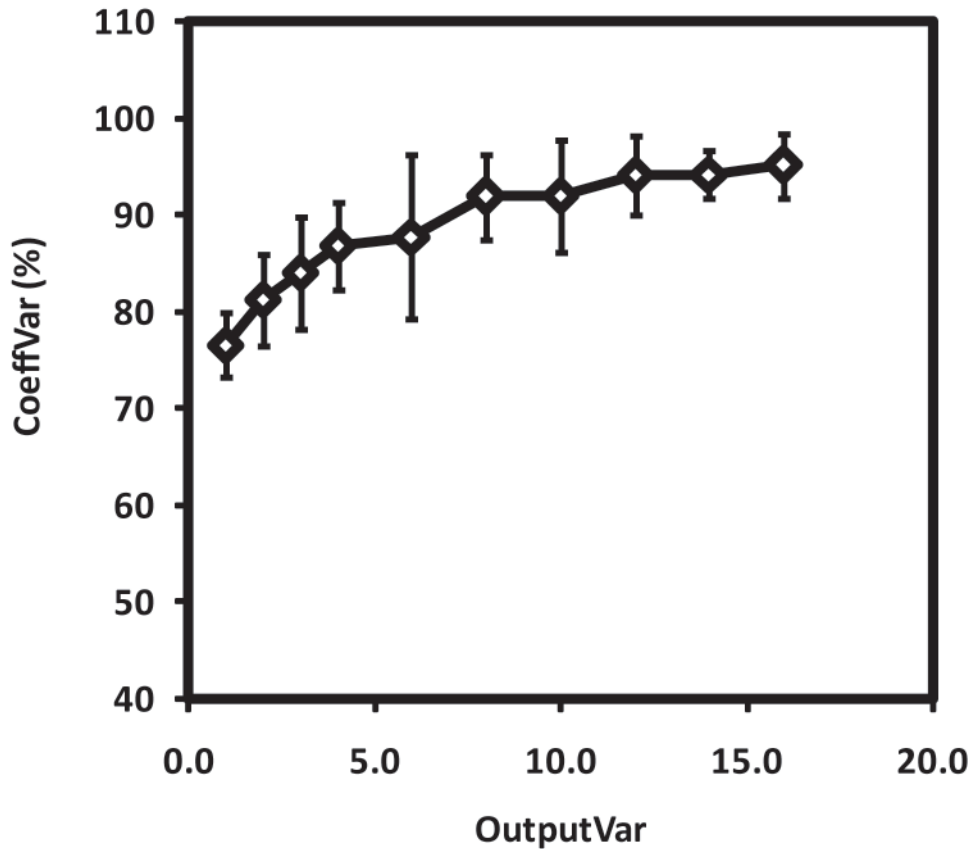


Supplementary Figure 5.2

Mean probability of a given trial exceeding the previous trial during the learning task

A trial-by-trial analysis was performed for each subject, session and block, measuring the probability of a given trial out-performing the previous trial in both the placebo (**A.**) and nicotine (**B.**) sessions. There was no overall increase in this probability across the 6 blocks, suggesting that no underlying rule governing successful performance in the task was being exploited by subjects. Further, within the placebo arm there was no difference in this probability between the sham and

theta burst sessions, suggesting that were any such rule-based process being exploited it would not account for the improved task acquisition observed following theta burst.



Supplementary Figure 5.3

Coefficient of Variation applied to learning model

Coefficient of Variation as measured using analysis for Experiment 2 behavioural data (CoeffVar) as applied to our model simulation, testing various values of OutputVar. The positive correlation between CoeffVar and OutputVar supports the method of analysing CoeffVar.

Chapter 6

Learning a new motor skill after stroke: effects of levodopa and brain stimulation

Work described in this chapter is being prepared for submission:

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6.1 Introduction

Non-invasive brain stimulation and pharmacological interventions represent promising therapeutic strategies for improving motor function after stroke, but so far the holy grail of this work – robust, sustainable improvements – remains elusive. Transient performance improvements can be induced by modulating activity in the motor cortex of either the lesioned (Hummel & Cohen 2005; Talelli et al 2007) or contralesional (Mansur et al 2005; Fregni et al 2006) hemisphere, but more recently the focus has moved to combining stimulation (Celnik et al 2009; Emara et al 2010; Khedr et al 2005; Kim et al 2006; Koganemaru et al 2010) or pharmacological interventions (Floel et al 2005a) with motor training, with some encouraging results. Such interventions are thought to engage mechanisms of neural plasticity similar to those involved in motor learning, and the limited success in some cases may reflect a homeostatic interaction between stimulation / drug intake and subsequent learning (see Bolognini et al 2009 for review). The process of motor learning may be regarded as having distinct phases: online changes (during training), offline changes (between the end of a training session and the start of the next) including reconsolidation, and longer-term retention / forgetting of gains (Luft & Buitrago 2005; Censor et al 2010). These different stages of learning are thought to be subserved by different mechanisms, and are known to respond differently to interventions aiming to perturb or enhance training effects in healthy subjects (Muellbacher et al 2002; Reis et al 2009). Finding ways to extend the application of novel therapeutic interventions from single to multiple sessions will be crucial in developing treatments that result in cumulative clinical benefit. This would be easier if one could first identify

the specific stages of motor learning facilitated by stimulation techniques or dopaminergic agents over the course of a single session.

Here we tested the effects of Theta Burst Stimulation (TBS) and dopaminergic stimulation on subsequent motor training in a group of patients with chronic stroke. TBS is a non-invasive technique that can induce a transient increase in cortical excitability lasting approximately 15 minutes (Huang et al 2005; Di Lazzaro et al 2008), and can enhance subsequent learning of a motor task in healthy humans (Teo et al 2010) consistent with other facilitatory effects of TMS on motor memory formation (Butefisch et al 2004). Dopaminergic agents are of interest because of demonstrated benefit in human models of motor learning and synaptic plasticity (Floel et al 2005b; Meintzschel & Ziemann 2006; Kuo et al 2008a), and in motor function after stroke in some but not all studies (Berends et al 2009; Scheidtmann et al 2001; Floel et al 2005a). We combined the 2 interventions as evidence has recently emerged that pharmacological modulation of neuroplasticity induction can in some cases enhance or prolong the resulting physiological changes (Butefisch et al 2002; Nitsche et al 2004b; Kuo et al 2008a; Swayne et al 2009). By re-testing task performance at a number of time points after completion of training we were able to dissociate the effects of these 2 interventions on immediate task performance, online changes during training, offline changes and retention of performance gains up to one week.

6.2 Methods

6.2.1 Participants

12 patients with chronic ischaemic stroke causing hand weakness participated in the study (age 62.5 ± 3.4 , mean \pm SE; 3 female; 1 left-handed). A minimum of 6 months had elapsed since stroke at the time of recruitment (see Table 6.1 below for details).

Participants were recruited from patient databases at 2 sites: the National Institute of Neurological Disorders and Stroke (NINDS, National Institutes of Health, Bethesda, Maryland, USA: 9 patients) and the National Hospital for Neurology and Neurosurgery (NHNN, London, UK: 3 patients). Patients had strokes not involving motor cortical regions (on review of Magnetic Resonance Imaging) with an initial severe paresis in the affected upper limb (MRC grade 2 or less) and considerable motor recovery (minimum grade 4 for thumb abduction).

6.2.2 Study design

The experimental protocol is shown in Figure 6.1. Patients each participated in 4 sessions in a randomised, placebo-controlled cross-over trial. For each session patients took either Carbidopa-Levodopa (100mg-25mg) or a placebo preparation (starch powder and magnesium stearate in a pink opaque capsule: both preparations dispensed and packaged by hospital pharmacy) and received either genuine or sham Theta Burst Stimulation (TBS) in a 2x2 design.

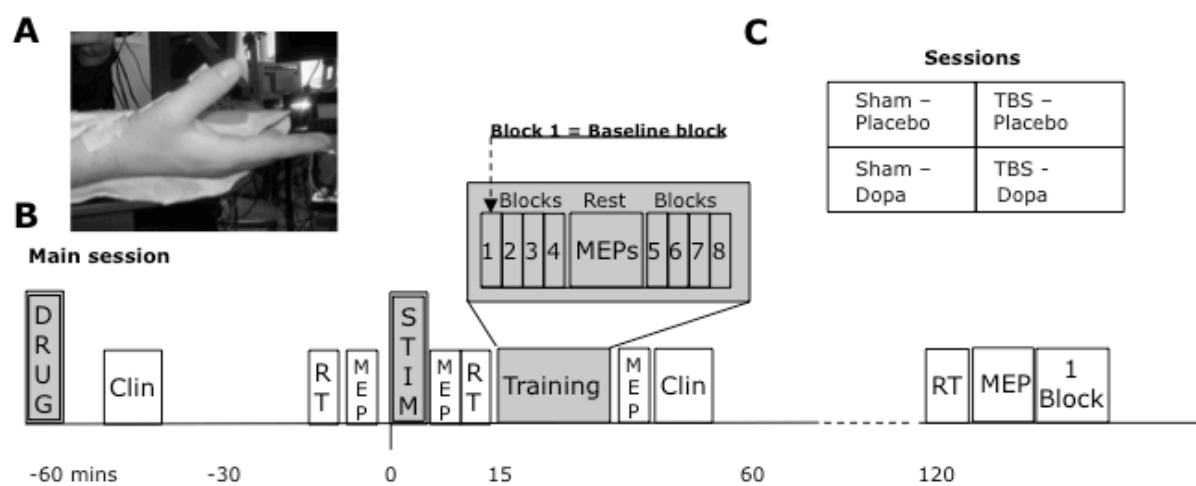


Figure 6.1. Experimental protocol

Movements were detected by an accelerometer on the proximal phalanx of the thumb (affected side), with online visual feedback of performance improvements (A). Each block comprised 60 movements in response to an external auditory cue at 0.5 Hz. In each main session patients received L-Dopa (or placebo) 60 minutes prior to Theta Burst Stimulation (TBS, real or sham). Training started approximately 10 minutes after stimulation and consisted of 8 blocks. Assessments of clinical scores of upper limb function (Clin) and simple reaction times (RT) were acquired as shown, and one further block was measured at 2 hours (B). Patients each participated in 4 sessions (C) in a randomised, placebo-controlled, counter-balanced crossover design.

A cross-over rather than factorial design was chosen in order to maximise the statistical power achievable with the patients recruited and to minimise the influence of patient heterogeneity on any observed effects resulting from the interventions. Sessions were separated by a minimum of a week, significantly exceeding the effects of both L-Dopa

(peak plasma concentration and physiological effects at 60 minutes: Floel et al 2005b; Kuo et al 2008a) and TBS (modulation of excitability lasting approximately 15 minutes; Huang et al 2005). With regard to the training task we would not expect performance gains to be retained after this interval, which exceeds the effective washout period employed previously for a ballistic non-sequential thumb movement task (Muellbacher et al 2002) and our own preliminary data. In order to be more confident of this we first acquired pilot data from 10 healthy subjects (9 female, Age 29.1 ± 5.4 (Mean \pm SD)) who performed the same ballistic thumb task in the absence of any intervention. Each subject trained in the task in 2 sessions separated by 1 week. A 2-way ANOVA revealed a significant effect on peak acceleration of Block ($F=8.3$, $P=0.018$) but no main effect of Session ($F=1.4$, $P=0.274$) nor Session x Block interaction ($F=0.04$, $P=0.841$) suggesting no retention of performance gains across the 1 week interval. In the patient experiments the session order was counter-balanced for sessions 1 to 3 with Sham-Placebo for session 4, so that any unexpected systematic effects of session order would be conservative for the active interventions.

Sessions were timed so that there was an interval of 60 minutes between taking medication (or placebo) and receiving TBS (or sham stimulation), coinciding with peak plasma concentration and demonstrable physiological effects in humans (Floel et al 2005b; Kuo et al 2008a). The investigator conducting the session left the room at the time of stimulation, remaining blind to session type. The first training block started 10 – 15 minutes after the completion of stimulation. In addition to training in the ballistic thumb movement task, patients were assessed for corticospinal excitability, simple reaction

times and clinical assessments of upper limb function. Follow-up values of these measures were acquired for each session at 2 hours post-stimulation (except for clinical assessments), on Day 2 and at 1 week. Patients completed a self-rating questionnaire at 2 points within each session: before the first reaction times block (after drug, before stimulation) and shortly after completing the main training session. These provided a subjective rating of expectation from the 2 interventions (before training), perception of any effect of the interventions on performance and the degree of encouragement provided by the investigator (after training). Patients also rated a number of emotional indices at both time points: fear, confusion, sadness, anger, tiredness, happiness, tension and alertness. These questionnaires were included in order to test for effects of the interventions on emotional state and to ensure that investigators were not influencing the outcome of training by non-verbal cues.

6.2.3 Transcranial Magnetic Stimulation

For active motor threshold measurement and TBS a Magstim Rapid stimulator was used (Magstim Co., Dyfed, UK). The stimulator was connected to a figure-of-eight coil with an internal wing diameter of 70 mm, held with the handle pointing posterolaterally. Electromyographic (EMG) recordings were made using a belly-to-tendon montage from the first dorsal interosseous muscle (FDI) in the affected upper limb. The raw signal was amplified and filtered with a band-pass filter of 50 Hz to 2000 Hz (Dantec Electronics, Skovlunde, Denmark), digitized at 2 kHz (CED Power1401, Cambridge Electronic Design, Cambridge, UK) and stored on a computer for offline analysis. The location of the FDI hand representation in the affected hemisphere was determined and the active

Motor Thresholds measured: see chapter 2 for details. MEP amplitudes were recorded in blocks of 15 consecutive stimuli delivered at 0.2 Hz. Before TBS a baseline block was recorded using a stimulus intensity adjusted to evoke an MEP of 1 mV amplitude in the contralateral FDI, which was kept relaxed throughout: this intensity was then used for the post-stimulation block. The stimulus intensity was then re-adjusted for a target amplitude of 1 mV (to account for any changes in MEP amplitudes following TBS) and this new intensity was used for all remaining blocks in that session.

Theta Burst Stimulation was given according to the intermittent (iTBS) protocol described by Huang et al (2005): see Chapter 2. This stimulation protocol has been shown to produce an increase in corticospinal excitability lasting up to 15 minutes (Huang et al 2005). For sham stimulation, a custom sham coil (Magstim) was used which generates equivalent noise and vibration without magnetic stimulation.

6.2.4 Ballistic thumb movement task

We used a modified version of a well-characterised task in which subjects practise a ballistic thumb movement aiming to increase peak acceleration (Muellbacher et al 2001, 2002). The patient's paretic hand was positioned supine on a board with the wrist, metacarpophalangeal and distal inter-phalangeal joints fixed with Velcro straps. The thumb was left unsecured and could abduct and oppose freely. An Endevco model 25A Isotron monoaxial accelerometer (Endevco Corporation, San Juan Capistrano, California; sensitivity 5 mV/g) was attached on the lateral aspect of the thumb's proximal phalanx with the maximal vector in the plane of thumb abduction. The accelerometer signal was

sampled at 2000Hz and not filtered. Training started 10 minutes after the completion of stimulation. Patients were asked to perform ballistic thumb abduction movements in time with a 0.5Hz audio metronome, with the explicit aim of maximising the initial peak acceleration. This movement was chosen as it is less natural than a pinch and may therefore have greater scope for change in response to training. The computer monitor provided online visual feedback, displaying the most recent movement trace with superimposed cursors showing the mean and best accelerations for that block in order to aid motivation. In a given session subjects performed 8 training blocks, each consisting of 60 training movements and lasting 2 minutes. Training blocks were separated by rest periods of 1 minute: after block 4 there was a rest period of 3 minutes during which a block of MEP amplitudes was recorded. Previous experience with this paradigm has shown that improvements occur rapidly within the first training block (Teo et al 2010). As our primary interest was the effects of the interventions on training-related changes (rather than the documented effects on immediate motor performance) we used the early trials of Block 1 (see analysis) as our measure of baseline performance, rather than risk a change in performance occurring in the course of a separate baseline block. We were able to assess effects of the interventions on this baseline measure due to the repeated measures design.

6.2.5 Reaction Times

For simple reaction times patients were asked to make a thumb abduction movement as soon as possible after an auditory tone, with the emphasis on an early response rather

than a large movement. Each block consisted of 20 trials with an auditory ‘go’ tone delivered at an average of 0.2 Hz with 20% variance of the inter-trial interval.

6.2.6 Clinical assessment of upper limb motor function

At each clinical assessment patients sat comfortably at a table and were scored in the Jebsen-Taylor Test (JTT) and Nine Hole Peg Test (NHPT): see Chapter 2 for details.

6.2.7 Data analysis and statistics

Motor thresholds were expressed as a percentage of maximum stimulator output. As a result of a technical failure the clinical scores were irretrievably lost for patient 10: the statistical analysis for clinical scores thus omits this patient. For the ballistic thumb movement task accelerometer traces were analysed offline. A custom script (Signal software, CED design Ltd, Cambridge, UK) was used to identify the peak of the first positive deflection in the plane of interest: this was adjusted manually for every trial to ensure precision and the peak accelerations for each trial recorded. Following a post-hoc analysis of within-block changes in task performance (see 6.3.1.1 below) the first 2 trials of each block were discarded as indicative of warm-up in each block and the subsequent 5 trials taken as representative of peak performance for that block: these 5 trials were treated as separate observations. For statistical analysis peak accelerations were expressed as logarithm base 10 in order to reduce the impact of clinical heterogeneity. For reaction times accelerometer traces were similarly analysed offline and defined as the interval between auditory cue and the first deflection: the mean reaction time was determined for each block.

For each measure multivariate repeated-measures ANOVA was used to test for the effects of ‘Time’ (or ‘Block’), ‘Intervention’ (all 4 session types) and their interactions. For the ballistic thumb movement task, changes in peak acceleration during the course of the main training session were tested across all 8 blocks while post-training changes were tested as part of an ANOVA incorporating 4 time points (training Block 8, post-training 2 hours, Day 2, and 1 week). Changes in performance between time points of interest were determined for each patient and session type as simple differences. The main training session was sub-divided in this way into early (Blocks 1 to 2) and late (Blocks 2 to 8): see Results. Post-training changes were determined as the differences between training Block 8 and the 3 subsequent time points (2 hours, Day 2 and 1 week). Where ANOVA revealed an effect of session type post-hoc paired t-tests were used to test differences. The SPSS 12.0 package was used for performing statistical analysis.

6.3 Results

Patients’ clinical details and baseline clinical scores are given in Table 6.1 (for medical history and medications see Supplementary Table 6.1). No side-effects were reported relating either to the active medication or to Theta Burst Stimulation (TBS). One patient reported mild transient scalp discomfort during single pulse TMS measurements. The rate at which patients correctly identified which interventions they had received was 54.6% for active medication vs placebo and 65.9% for TBS vs sham stimulation. Neither of these was significantly different from chance (Fisher’s exact test: $P=0.763$ for placebo vs L-Dopa, $P=0.055$ for TBS vs sham), suggesting that blinding was effective.

Questionnaires revealed no differences between session types in patients' perceptions of the degree of encouragement offered by the investigator, investigator competence, expectations (beforehand) or perceptions (afterwards) of whether interventions affected performance, nor in the 8 emotional indices recorded (see Methods, $F_{3,9} < 3.0$, $P > 0.05$ all ANOVAs).

Patient (years)	Age	Sex	Months since stroke	Lesion site	JTT score (seconds)	NHPT score (%)
1	79	F	48	L temporal lobe / insula	51.7	36.7
2	66	M	39	R caudate nucleus	36.3	93.6
3	45	M	64	R pons	31.0	60.4
4	46	M	354	R Basal Ganglia / centrum ovale	26.5	68.7
5	75	M	58	L MCA and posterior watershed zones	50.8	43.9
6	57	M	81	R parietal cortex / insula	77.6	14.4
7	82	M	105	R Basal Ganglia	57.6	22.7
8	61	F	11	R internal capsule	44.7	69.8
9	59	M	109	R globus pallidus / internal capsule	27.0	78.1
10	63	M	12	L internal capsule	*	*
11	60	M	33	R MCA (sparing cortex)	33.8	67.8
12	57	F	21	L internal capsule	28.3	75.9

Table 6.1. Patient characteristics and baseline clinical scores

(L = Left; R = Right; MCA = Middle Cerebral Artery; * data unavailable, see 6.2.7 above)

6.3.1 Ballistic thumb movement task

6.3.1.1 Performance within individual blocks

In order to determine a suitable measure to reflect optimum task performance a trial-by-trial analysis of the behavioural data was undertaken. Visual inspection suggested that performance tended to decline in the course of each block, presumably with fatigue, with subsequent recovery and some improvement occurring between blocks (Figure 6.2A).

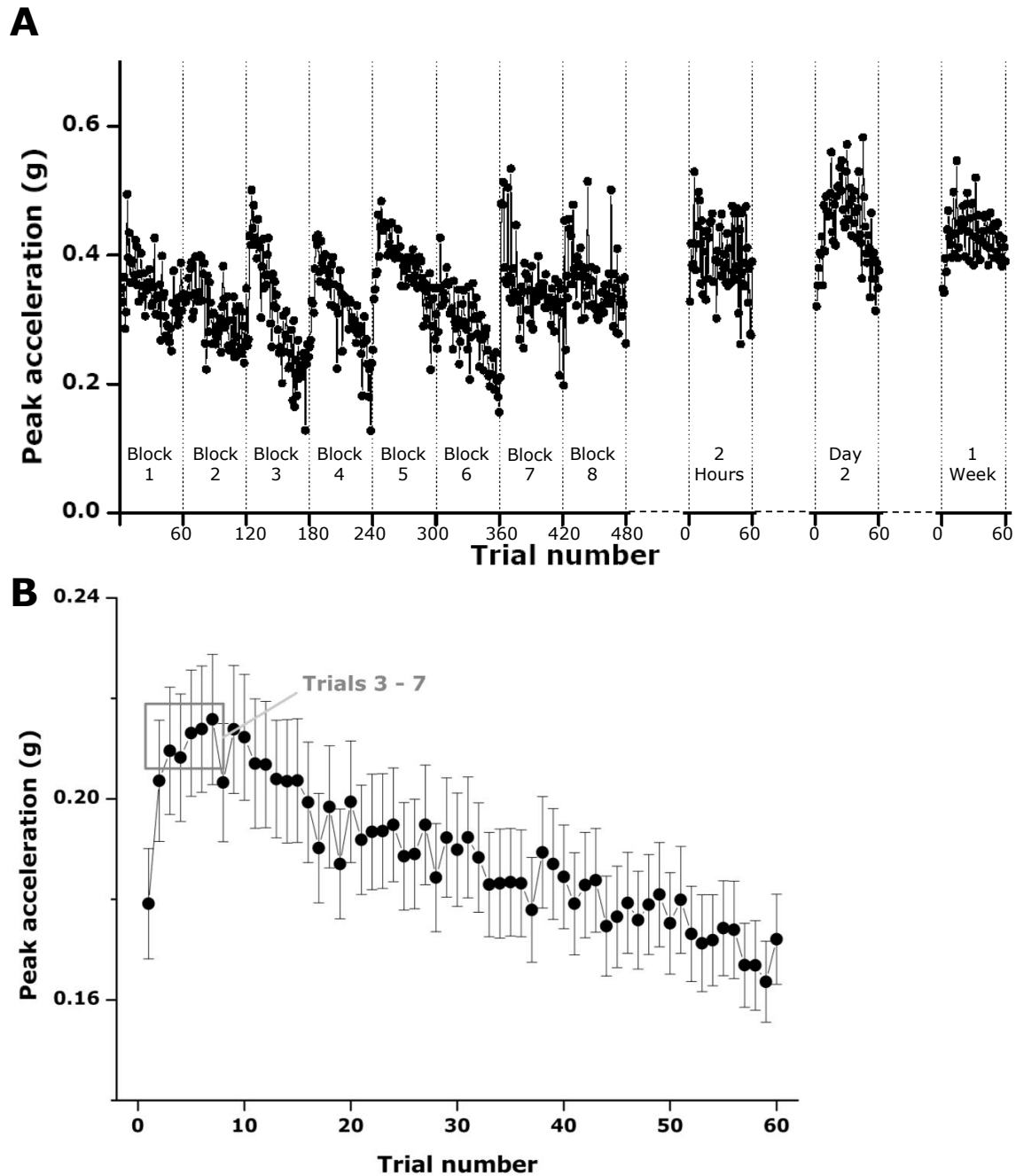


Figure 6.2 Within-block changes in task performance

A. Peak acceleration is shown for every trial performed by a single representative patient, including follow-up blocks (Sham-Placebo condition). Within a given block a rapid improvement was observed over the first few trials followed by a subsequent decline, presumably due to fatigue.

B. A trial-by-trial analysis incorporating every block, condition and patient confirmed that this pattern of early improvement with subsequent decline was observed across the group as a whole (mean + SE shown). Comparison of the first 10 and last 10 trials revealed a significant decline (paired t-test, $P < 10^{-12}$). On the basis of this pattern of performance we chose the 5 consecutive trials starting from trial number 3 as providing a representative measure of peak performance within a given block.

We determined the mean peak acceleration separately for each of the 60 trials within an average block (all patients, session types and blocks, Figure 6.2B) and this confirmed a pattern of initial improvement over the first 2 trials followed by steady decline.

Comparison of the first 10 vs last 10 trials within a given block demonstrated a highly significant drop in peak acceleration (paired t-test, $P < 10^{-12}$). On the basis of the observed pattern we chose to discard the first 2 trials (as ‘warm-up’ trials) and to take the mean of the subsequent 5 trials - trials number 3 to 7 - as a representative measure of optimum performance before fatigue. We analysed the following aspects of task performance separately: pre-training performance (immediately after stimulation and before training occurs), changes during the training session and changes relative to the end of training.

6.3.1.2 Pre-training task performance

Mean peak acceleration immediately after drug and stimulation (but before training) differed significantly between session types (ANOVA: effect of Intervention, $F_{3,57}=3.7$, $P=0.017$) with best performance observed in the context of both active interventions combined (TBS-Dopa sessions). Post-hoc t-tests revealed that immediate pre-training

performance with the combined active interventions was significantly better than that with either intervention alone (paired t-tests vs TBS-Placebo $P=0.017$; vs Sham-Dopa $P=0.010$). Pre-training performance in a given session was not affected by previous session type (no effect of Intervention), providing reassurance that gains were not carried over between sessions.

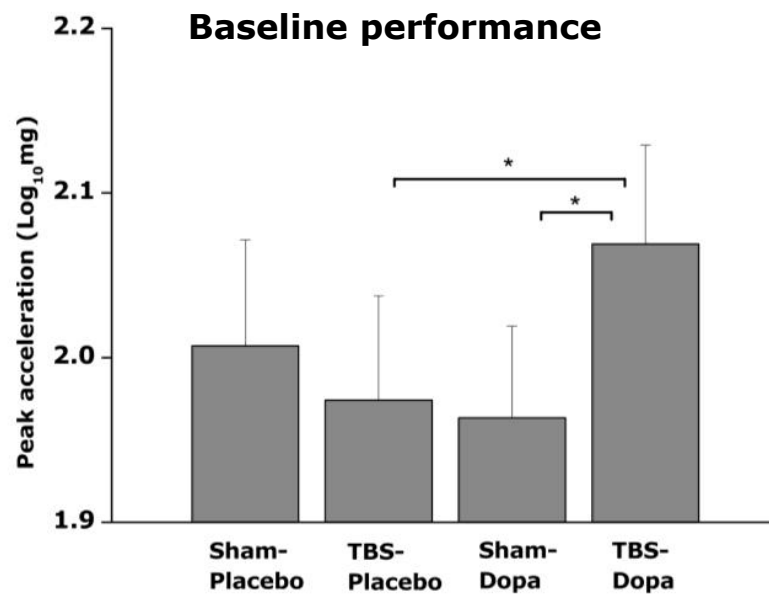


Figure 6.3. Effect of interventions on pre-training task performance

Mean peak acceleration (+ SE) is shown for the first training block within each session type, as a measure of baseline task performance after administration of TBS/sham and Dopa/placebo but before training occurs. There was a significant effect of Intervention on initial performance (see text for ANOVA details). Best initial performance was observed in sessions with both active interventions (TBS and Dopa). Performance in these sessions was significantly better than with either intervention individually (* $P<0.05$: see text for details).

6.3.1.3 Changes in task performance during training session

A 2-way ANOVA with the factors Intervention and Block revealed that patients improved significantly over the course of training (main effect of Block, $P < 0.001$) and that the time course of performance change differed between session types (Intervention x Block interaction, $P < 0.001$ – see Table 6.2 for ANOVA details).

Main ANOVA factors	Degrees of Freedom; error	Effects	F	P
<u>Performance across 8 Blocks</u>				
2 way I x B	21; 39	I x B	3.6	<0.001
	3; 57	I	1.6	0.207
	7; 53	B	14.3	<0.001
<u>Total change within session: Block 1 to Block 8</u>				
1 way I	3; 57	I	8.7	<0.001
<u>Early changes: Block 1 to Block 2</u>				
1 way I	3; 57	I	4.0	0.013
<u>Late changes: Block 2 to Block 8</u>				
1 way I	3; 57	I	6.4	0.001

Table 6.2. ANOVAs for training session (B, Block; I, Intervention)

The total improvement within the training session (Block 1 to Block 8: Figure 6.4B) differed between session types (significant effect of Intervention at ANOVA). Post-hoc t-tests revealed that there was less improvement with the combined active interventions (TBS-Dopa) than with either of the placebo session types (paired t-tests: Sham-Placebo >

TBS-Dopa $P < 0.01$, TBS-Placebo $>$ TBS-Dopa $P < 0.001$). This is likely to reflect the fact that in the TBS-Dopa sessions patients started from a higher pre-training level of performance.

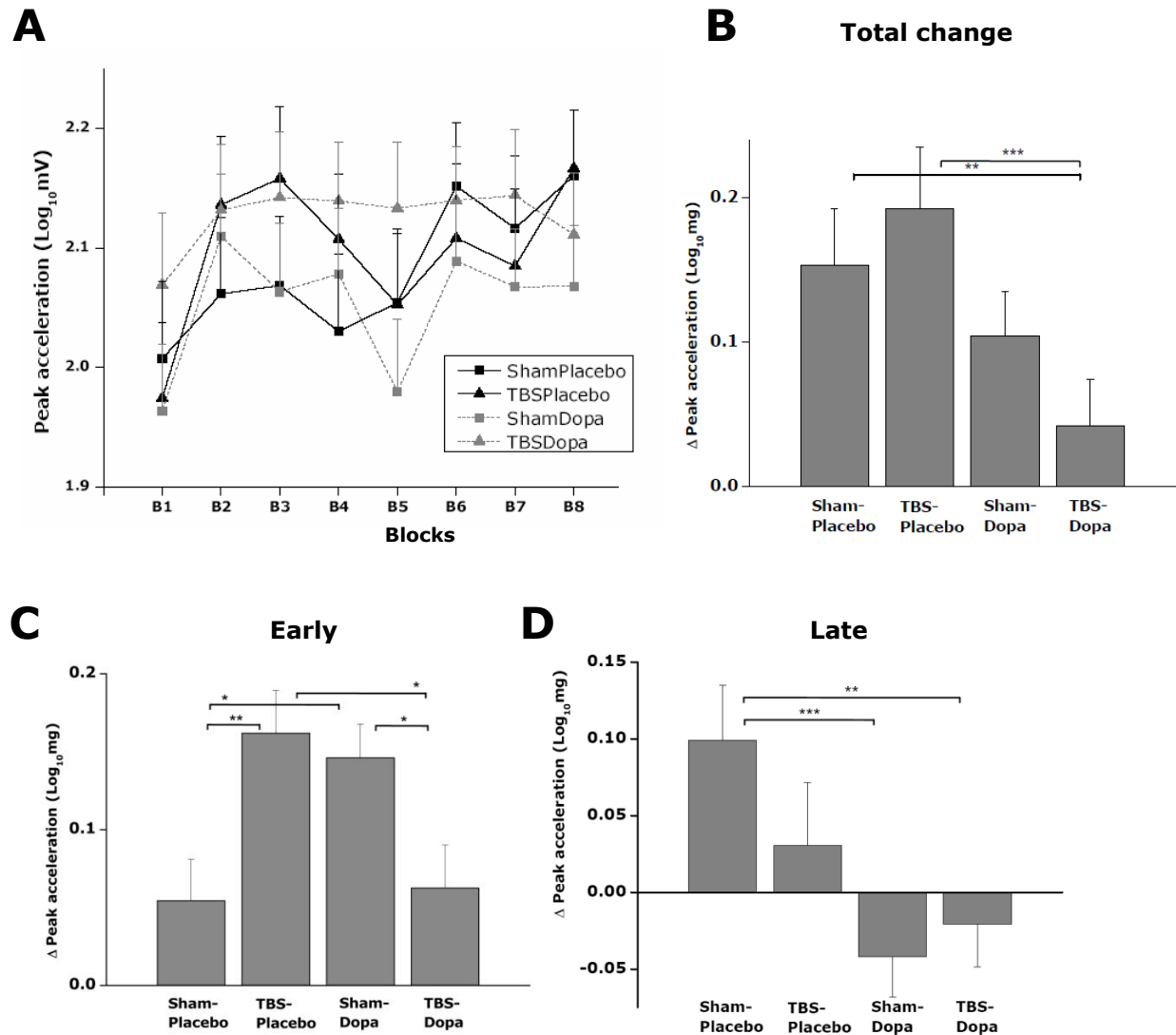


Figure 6.4. Changes in task performance during training session

A. Mean peak acceleration (\pm SE) is shown for each session type. There was significant improvement in performance with training as a whole (main effect of Block). The time course of performance change differed between session types, with a significant Intervention \times Block interaction (see Table 6.2 for ANOVA details).

B. Overall change in performance within the session (Block 1 to Block 8).

Differing degrees of change were observed between session types (significant effect of Intervention). Least improvement was observed following the combined active interventions.

C. and D. Changes in performance are shown separately for 2 phases: early training (Block 1 to Block 2, **C**) and subsequent training (Block 2 to Block 8, **D**). The changes observed in both phases differed between session types (significant effects of Intervention). Greatest early improvement was observed with either intervention given individually, and less change was observed with no intervention or with both given in combination. During subsequent training there is greater improvement in the Sham-Placebo condition than with either or both interventions. At this stage there is little further improvement with TBS alone, and a slight decline in performance with Dopa alone or with combined interventions. Overall, steady improvement was observed in the absence of any intervention: with either intervention alone, there was accelerated improvement in the early phase but this rate of change was not sustained (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

In order to investigate the differing time course of performance changes we divided training into early and late, with Block 2 as the midpoint in view of the marked changes observed between Blocks 1 and 2 (Fig 6.4A). We calculated the performance changes across these 2 phases in individual patients. ANOVAs revealed that the extent of change differed according to session type in both phases (significant effects of Intervention – see Table 6.2). In the early training phase (Figure 6.4C) the greatest improvements were seen with either active intervention given individually (TBS-Placebo or Sham-Dopa) but not in combination (TBS-Placebo > TBS-Dopa, Sham-Dopa > Sham-Placebo, Sham-Dopa > TBS-Dopa all $P < 0.05$; TBS-Placebo > Sham-Placebo $P < 0.01$). By contrast, in the late training phase (Figure 6.4D) the greatest improvements were observed in the Sham-

Placebo sessions, with little further change or slight decline in other session types (Sham-Placebo > TBS-Dopa $P < 0.01$; Sham-Placebo > Sham-Dopa $P < 0.001$). Overall, these results suggest a different time course of training-dependent changes in performance depending on the pre-learning interventions.

6.3.1.4 Changes in task performance following completion of training

We tested changes in performance across 4 time points: completion of training (Block 8), 2 hours, Day 2 and 1 week (Figure 6.5A below). Performance as a whole changed significantly following training (main effect of Block) but the time course differed according to session type (significant Intervention x Block interaction, no main effect of Intervention – see Table 6.3 for ANOVA details).

Between the completion of training and the 2 hour follow-up assessment there was a significant decline in performance across all session types (2-way ANOVA across 2 blocks: significant main effect of Block, $F_{1,59}=30.5$, $P < 0.001$; no Intervention x Block interaction). Testing the differences (Block 8 to 2 hours) revealed no interactions or main effects (see Table 6.3), suggesting that performance declined to a similar extent regardless of intervention.

By contrast, the overnight change in performance by Day 2 (Block 8 to Day 2: Figure 6.5B below) differed between session types (significant effect of Intervention). Although there was no overall change across this interval (2-way ANOVA: no effect of Block, $F_{1,59}=1.9$, $P=0.178$), offline improvement was observed in the Sham-Dopa sessions whereas performance declined in other session types (paired t-tests Sham-Dopa vs other session types all $P < 0.01$).

Main ANOVA factors	Degrees of freedom; error	Effects	F	P
<u>After end of training: Block 8, 2 hours, Day 2, 1 week</u>				
2 way Intervention x Block	9; 51	I x B	5.0	<0.001
	3; 57	I	1.7	0.181
	3; 57	B	13.1	<0.001
<u>2 hour follow-up: Block 8 to 2 hours</u>				
1 way Intervention	3; 57	I	1.0	0.386
<u>Offline changes: Block 8 to Day 2</u>				
1 way Intervention	3; 57	I	7.7	<0.001
<u>Retention: Block 8 to 1 week</u>				
1 way Intervention	3; 57	I	3.3	0.026
<u>Overall change: Block 1 to 1 week</u>				
1 way Intervention	3; 57	I	3.1	0.032

Table 6.3. ANOVAs for performance after end of training (B, Block; I, Intervention)

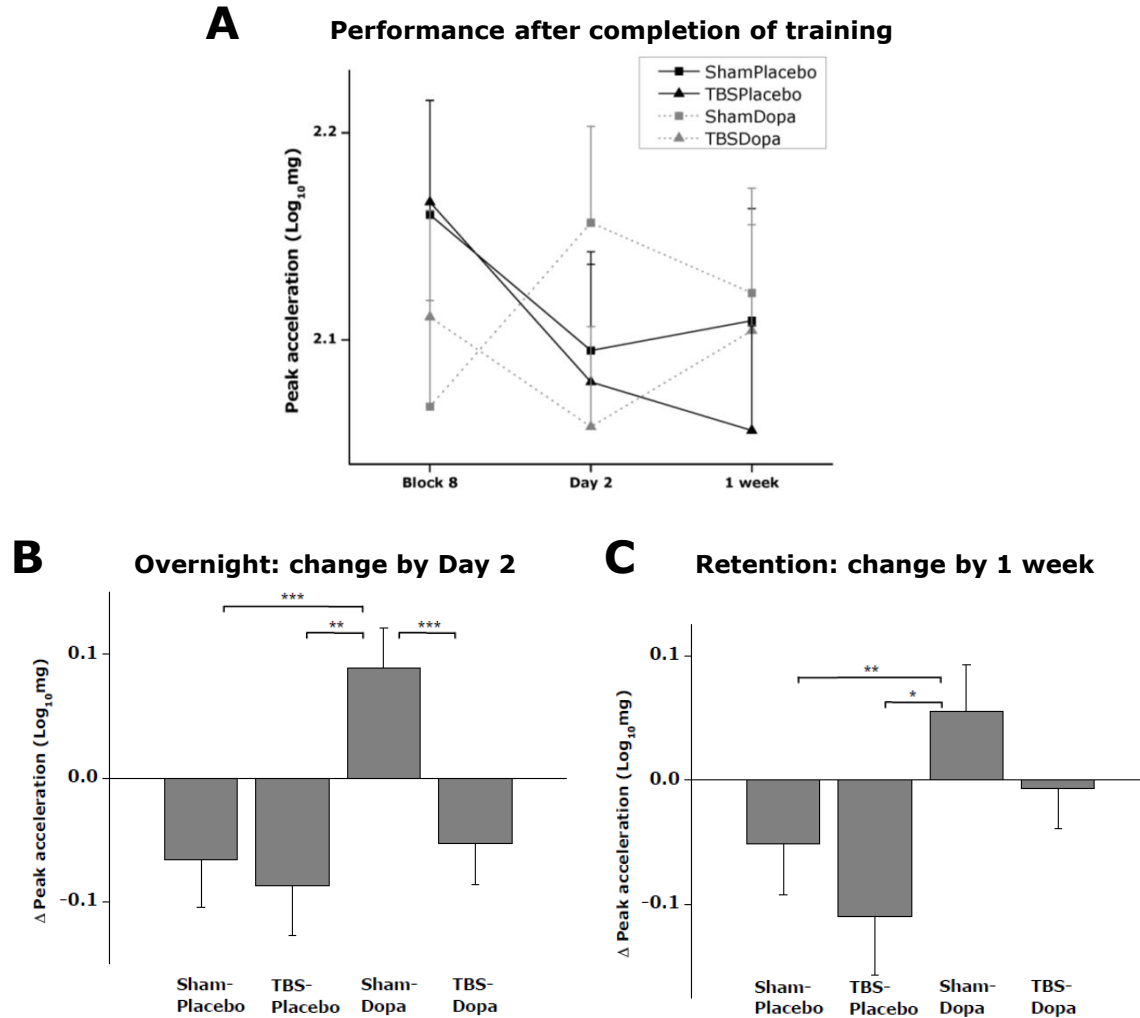


Figure 6.5. Changes in performance after completion of training

A. Task performance is shown from Block 8 to 1 week (omitting the 2 hours time point). Performance differed significantly between session types at Block 8 and Day 2 but not at 1 week.

B. Block 8 to Day 2. The change by Day 2 differed between session types (significant effect of Intervention), with marked offline improvement seen following Dopa alone but not in any other session type.

C. Block 8 to 1 week. The retention of performance gains by 1 week differed between session types (significant effect of Intervention).

Improvement across this interval was observed following Dopa alone but in no other session type (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

By 1 week (Block 8 to 1 week: Figure 6.5C) improvements were retained as a whole, with no overall change in performance (2-way ANOVA across 2 blocks: no effect of Block, $F_{1,59}=1.4$, $P=0.239$). On testing the change across this interval in different session types there was a significant effect of Intervention (see Table 6.3). There was improvement over this period only in the Sham-Dopa sessions with slight decline in other sessions (Sham-Dopa > Sham-Placebo, $P<0.05$; Sham-Dopa > TBS-Placebo, $P<0.01$).

6.3.1.5 Overall outcome of training

Between Block 1 and the 1 week follow-up there was improvement across session types (2-way ANOVA across 2 blocks: main effect of Block, $F_{1,59}=17.0$, $P<0.001$).

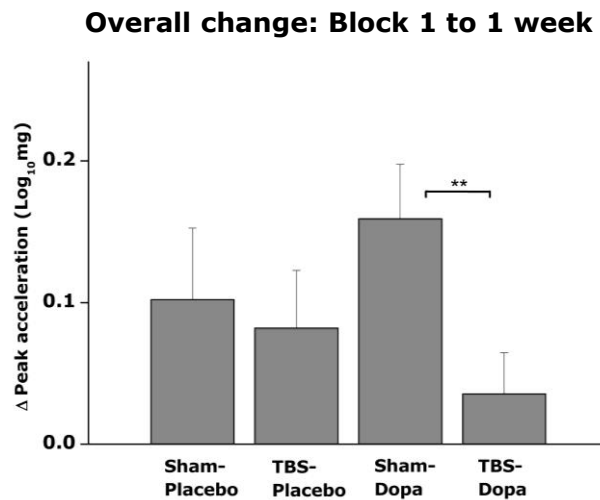


Figure 6.6. Overall outcome of training

The change from Block 1 to the 1 week follow-up assessment is shown. There was an overall improvement in performance across all session types, and the extent of this change differed between interventions (significant main effect of Intervention) with the greatest improvement observed with Dopa alone (** $P<0.001$, *** $P<0.0001$).

The change between these time points differed between session types (significant effect of Intervention – see Table 6.3). The greatest overall improvement was observed in the Sham-Dopa sessions, which were significantly more successful than the TBS-Dopa sessions (paired t-test, $P < 0.01$ uncorrected – see Figure 6.6). Despite this, however, performance at 1 week did not differ significantly between session types (no effect of Intervention).

6.3.1.6 Relating performance to subsequent changes

We tested the relationship between pre-training performance (Block 1) and total improvement across the training session (by Block 8) and found that across all 4 session types there was a negative correlation ($r = -0.54$, $P < 0.001$) such that patients with better baseline performance improved less during the session. Similarly, task performance at the end of training (Block 8) showed negative correlations with subsequent changes by Day 2 ($r = -0.36$, $P = 0.006$) and 1 week ($r = -0.36$, $P = 0.006$). The same relationship was observed for the overall outcome of training compared to Block 1 ($r = -0.57$, $P < 0.001$). Regardless of the intervention, therefore, changes in performance within and following training appeared to approach a natural asymptote. It is therefore worth considering whether apparent differences between session types in performance changes during and after training may in fact relate simply to differences at baseline. Performance differed between session types at the start of training (see ANOVA above), at completion of training (Block 8: effect of Intervention, $F_{3,57} = 3.4$, $P = 0.023$) and at Day 2 ($F_{3,57} = 5.4$, $P = 0.003$), but not at 1 week follow-up (NS). Of note is that while performance in the Sham-Dopa sessions was the poorest in Block 8 it was the best by Day 2 (see Figure

6.5A), suggesting that the overnight improvement observed in this group alone cannot be attributed to performance at the completion of training.

6.3.1.7 Summary of performance changes

The key finding is that we observed distinct effects of our interventions on the consecutive stages of motor learning in the ballistic thumb abduction task. The combination of TBS and Dopa resulted in greater peak acceleration immediately after stimulation. In the course of the training session patients significantly improved performance across all session types. Least improvement was observed with the combined active interventions, presumably due to starting from a higher level of pre-training performance. Compared to Sham-Placebo, either intervention on its own accelerated the effects of training without altering the outcome at the end of the session. By 2 hours performance had declined comparably in all session types. Significant offline improvement by Day 2 was observed in the context of Dopa alone, in which sessions performance was best at this stage, but not in other session types. By 1 week there was overall retention of performance gains: the overall improvement compared to Block 1 was greatest in the Sham-Dopa sessions, but final performance was comparable.

6.3.2 Reaction times

Mean reaction times for each session type are given in Table 6.4. There was no effect of any intervention on reaction times when testing Pre- and Post-Stimulation time points (Intervention x Time interaction, $F_{3,9}=1.2$, $P=0.358$; no main effects). On testing the 4 time points after stimulation, reaction times became shorter in the course of a given

session but the degree of change did not differ according to intervention (main effect of Time, $F_{3,9}=5.0$, $P=0.027$; no Intervention x Time interaction or main effect of Intervention).

	Reaction Time (ms)	Jebsen-Taylor Test score (s)	Nine Hole Peg Test (AH/UH%)
Sham- Placebo	137.1 ± 6.0	42.3 ± 1.5	57.5 ± 2.3
TBS- Placebo	148.0 ± 12.4	50.9 ± 2.3	58.0 ± 2.5
Sham- Dopa	143.2 ± 8.1	46.7 ± 1.6	57.0 ± 2.3
TBS- Dopa	145.4 ± 9.8	49.1 ± 2.0	55.3 ± 2.4

Table 6.4. Mean baseline values for Reaction Times and Clinical Scores

AH, Affected Hand; UH, Unaffected Hand; TBS, Theta Burst Stimulation
(The baseline Reaction Times and clinical scores were assessed after administration of Dopa / placebo and before stimulation / sham)

6.3.3 Clinical measures of upper limb function

Mean clinical scores for each session type are given in Table 6.4. For both the Jebsen-Taylor Test (JTT) and the Nine Hole Peg Test (NHPT), there was no overall change in scores over the course of a given session and no differences between session types (Intervention x Time ANOVAs : no Intervention x Time interactions, no main effects of either factor).

6.3.4 Electrophysiological measurements

6.3.4.1 Motor thresholds

Baseline resting and active motor thresholds are given in Table 6.5.

Patient	Resting threshold		Active threshold	
	UH	AH	UH	AH
1	29	38	25	27
2	49	50	45	42
3	37	33	42	42
4	48	45	56	51
5	46	48	51	52
6	36	62	48	70
7	33	65	41	75
8	25	56	42	44
9	52	55	46	50
10	30	35	24	25
11	40	36	43	42
12	44	52	54	59

Table 6.5. Baseline resting and active motor thresholds

Values given as % of maximum stimulator output

(UH = Unaffected Hemisphere; AH = Affected Hemisphere)

Resting thresholds were significantly raised in the affected hemisphere (AH) compared to the unaffected hemisphere (UH) (paired t-test, $P=0.042$) but this was not the case for active thresholds ($P=0.142$). Resting thresholds showed a significant positive correlation with baseline Jebsen-Taylor Test (JTT) scores and a trend negative correlation with Nine Hole Peg Test (NHPT) scores (JTT: $r=0.62$, $P=0.022$; NHPT: -0.46 , $P=0.077$). Active

thresholds showed significant equivalent positive and negative correlations respectively (JTT: $r=0.67$, $P=0.012$; NHPT: $r=-0.74$, $P=0.005$). Thus, as previously described, raised thresholds in the AH were associated with relatively greater functional impairment.

Motor thresholds were measured at 4 points in the course of each session (Supplementary Figure 6.1) and 3-way ANOVAs revealed that these did not change across these time points (rMT: Drug x Stim x Time interaction, $F_{3,8}=1.0$, $P=0.438$; main effect of Time, $F_{3,8}=0.36$, $P=0.341$. aMT: Drug x Stim x Time interaction, $F_{3,8}=2.7$, $P=0.116$; main effect of Time, $F_{3,8}=1.8$, $P=0.218$). For rMT there was a significant Drug x Stim interaction ($F_{1,10}=10.2$, $P=0.010$) and a trend main effect of Drug ($F_{1,10}=5.0$, $P=0.051$) suggesting a tendency for higher resting thresholds in the Dopa sessions. Active thresholds showed no equivalent Drug x Stim interaction or main effect of Drug ($F_{1,10}=1.2$, $P=0.294$; $F_{1,10}=0.03$, $P=0.857$ respectively).

6.3.4.2 Motor Evoked Potential (MEP) amplitudes

Baseline MEP amplitudes recorded before stimulation (but after Dopa / placebo), and those recorded before the start of training (but after stimulation), are given in Table 6.6. There was no change in amplitudes immediately following TBS, nor any effect of Dopa at these time points (3-way ANOVA, Drug x Stim x Time interaction $F_{1,11}=0.004$, $P=0.949$; no significant main effects). In relation to motor training (Pre, Midpoint, Post, 2 hours, Day 2, 1 week) there was a tendency for MEP amplitudes to increase (trend main effect of Time, $F_{5,7}=3.5$, $P=0.066$) but this did not differ between session types (no Drug x Stim x Time interaction, $F_{5,7}=0.61$, $P=0.700$). MEPs were larger in the Dopa sessions

compared to Placebo (main effect of Drug, $F_{1,11}=6.1$, $P=0.031$; no main effect of Stim) but there was no interaction of either Drug or Stimulation with Time.

	MEP pre-stimulation (mV)	MEP pre-training (mV)
Sham-Placebo	0.84 ± 0.18	0.72 ± 0.12
TBS-Placebo	0.68 ± 0.13	0.60 ± 0.09
Sham-Dopa	0.76 ± 0.13	0.74 ± 0.12
TBS-Dopa	0.78 ± 0.15	0.76 ± 0.14

Table 6.6. Mean baseline values for MEP amplitudes
MEP, Motor Evoked Potential; TBS, Theta Burst Stimulation

In order to allow for the possibility that the polyphasic morphology of evoked potentials influenced these outcomes MEP areas were also tested, with identical results (baseline values and statistics given in Supplementary Table 6.2).

6.4 Discussion

We observed distinct effects of our 2 interventions - Theta Burst Stimulation (TBS) applied to the contralateral motor cortex and dopaminergic stimulation - on the consecutive stages of performance and learning in the ballistic thumb movement task.

Our study design included measurements of pre-training performance, changes occurring within the training session (online), further changes occurring overnight (offline) and the longer-term stability of performance gains up to 1 week (retention). We found that both interventions given together resulted in better immediate task performance relative to the other interventions. Either one given individually accelerated online improvements during training, enhancing early gains but not altering the total within-session change. While the default overnight change was a slight deterioration in performance (offline forgetting), L-Dopa alone converted this to an offline improvement and performance at Day 2 was best in these sessions. There was little further change by one week, with final performance being comparable between session types.

6.4.1 The 2 interventions

The TBS protocol used here induces a transient increase in excitability of the stimulated motor cortex lasting around 15 minutes (Huang et al 2005). This effect is thought to be mediated by synaptic strengthening and is dependent on activity at the NMDA glutamate receptor, thereby resembling Long Term Potentiation (LTP) (Huang et al 2007; Teo et al 2007). The same protocol has previously resulted in an immediate improvement of simple reaction times in a group of chronic stroke patients (Talelli et al 2007), although lasting effects are as yet unknown. Although the effect of TBS on motor learning has not been formally tested in the context of stroke before, a recent study in healthy subjects has shown that it increases the rate of improvement in the same task as that employed here (Teo et al 2010), consistent with the effects of other forms of TMS on motor memory formation (Butefisch et al 2004).

Dopaminergic stimulation is recognised as having a beneficial effect in a number of learning paradigms, both motor (Meintzschel & Ziemann 2006; Floel et al 2005b; Floel et al 2008b) and non-motor (Knecht et al 2004; Reinholz et al 2009). In the motor system this has been ascribed to an improvement in the signal-to-noise ratio of synaptic transmission, with selective enhancement of activity at NMDA glutamate receptors at the expense of other excitatory receptors (Meintzschel & Ziemann 2006; Cepeda et al 1992). A recent study in rats has directly confirmed that dopaminergic stimulation has a positive effect on LTP within motor cortex, possibly by increasing intracellular calcium levels, and indeed is necessary for LTP to occur (Molina-Luna et al 2009). The influence of dopamine and its agonists on behaviour and on other plasticity paradigms is non-linear, with an inverted-U shape dose-response curve consistently described (Cai & Arnsten 1997; Seamans & Yang 2004; Monte-Silva 2009). A similar effect has also been demonstrated for artificial LTP induction (Kolomiets et al 2009). It is likely that the L-Dopa dose of 100 mg chosen here is at the top of this curve, as the same dose is known to stabilise synaptic changes resulting from paired associative stimulation (Kuo et al 2008a) and to promote motor memory formation in healthy subjects (Floel et al 2005b). Moreover, a similar beneficial effect was demonstrated in chronic stroke patients at the same dose (Floel et al 2005a). However, the effects of L-Dopa on the outcome of TBS are hitherto unstudied.

6.4.2 Pre-training task performance and online improvements

TBS alone did not induce higher pre-training peak accelerations here, consistent with the effect of TBS alone in healthy subjects (Teo et al 2010), but did so in combination with

L-Dopa relative to the other interventions. Studying stroke patients, Talelli et al (2007) observed an immediate improvement in reaction times but there are clear differences in the nature of these 2 motor tasks. In order to generate maximal thumb acceleration in the deliberately awkward direction of abduction, subjects must generate a motor output that favours task-relevant agonists at the expense of other motor units. Thus motor output selectivity is important in this task and it may be that a combination of enhanced synaptic activity (TBS) and an improved signal-to-noise ratio (L-Dopa) results in better pre-training performance relative to other interventions. Some investigators have reported that L-Dopa reduces motor cortical excitability, although this has not been a consistent finding, and one might speculate that this would have an adverse effect on performance. In the current study L-Dopa was in fact associated with slightly larger MEP amplitudes, although there was also a rise in resting motor thresholds. However a number of recent studies have observed dissociations between the effects of interventions on behaviour and cortical excitability (Reis et al 2009; Teo et al 2010), sounding a note of caution when trying to explain behavioural changes in terms of changes in excitability. This is particularly the case given that behavioural changes in, for example, motor memory formation are associated with opposite excitability changes in different muscle groups that operate as agonist or antagonist to the training task (Butefisch et al 2004; Duque et al 2008).

Patients improved performance of the peak acceleration task across the 8 training blocks in all session types. Such improvements must presumably involve a gradual change in the configuration of the motor output generated at each trial, with small performance

variations and the subsequent stabilising of beneficial changes. The latter part of this process is likely to involve synaptic strengthening within the motor cortex, a region which has been implicated previously in a similar training paradigm (Muellbacher et al 2001), and there is evidence to suggest that LTP has a role to play in forms of human motor learning (Butefisch et al 2000; Ilic & Ziemann 2005; Ziemann et al 2006; Fritsch et al 2010). The observation of faster improvements in this task following TBS alone is consistent with previous findings in healthy subjects (Teo et al 2010). It seems reasonable that enhanced NMDA glutamate receptor activity in the stimulated motor cortex may improve the induction of LTP in the context of training, leading to more rapid behavioural gains. A similar mechanism may be envisaged for the early effects of L-Dopa. Dopaminergic stimulation facilitates LTP induction in a number of brain regions, but in the context of this training paradigm enhanced LTP within the motor cortex would seem likely.

The lack of any enhancement of early training over placebo with both interventions given in combination is interesting and we can see 3 potential approaches to explaining this finding. First, it may be that the enhanced pre-training task performance observed with the combined interventions represented an accelerated form of the within-session changes, with a ceiling effect limiting further gains. This would suppose a similar physiological mechanism for behavioural changes occurring before and during training, which perhaps seems unlikely. However it is conceivable that increased excitability in the motor output system could enhance both pre-training performance and subsequent training-related gains, so a ceiling effect cannot be excluded. Secondly one could argue

that in the context of both active interventions the relative reduction in early gains represents a homeostatic response, with the previous administration of L-Dopa altering the subsequent response to TBS. In most existing models of such homeostatic changes the recent activity in relevant synapses determines the response to subsequent plasticity induction (Siebner et al 2004; Bienenstock et al 1982): an increase in synaptic activity would be beyond the known effects of L-Dopa, which is furthermore known to enhance subsequent LTP induction within the motor cortex (Molina-Luna et al 2009), making such an effect seem perhaps unlikely. Furthermore, attempts to demonstrate homeostatic mechanisms for human motor learning suggest that their impact in such a context is limited (Kuo et al 2008b). Thirdly, the limited gains with the combined interventions may be explained in terms of the non-linear effects of dopaminergic stimulation, where at higher doses the positive effects on behaviour or plasticity induction are lost or reversed (Cai & Arnsten 1997; Seamans & Yang 2004; Monte-Silva 2009). L-Dopa may promote LTP by raising intracellular calcium levels (Molina-Luna et al 2009) but with further increases activation of potassium channels may cause hyper-polarisation, limiting further changes (Misonou et al 2004). It is conceivable that with the combination of TBS and L-Dopa a similar phenomenon may limit the outcome of early training, but such mechanistic considerations are necessarily speculative.

6.4.3 Offline changes and the retention of performance gains

Following the completion of training, performance declined in all session types by 2 hours, and the default overnight change (sham-placebo) was a modest decline, insufficient to return performance to pre-training levels. Such an offline decline is

commonly observed in the context of skill learning, when it is referred to as the ‘warm-up decrement’ (Adams et al 1961). Under L-Dopa alone there was by contrast a marked overnight improvement. Changes in performance after completion of training may be influenced by the training schedule used (Tanaka et al 2010), and offline improvements may be induced by anodal direct current stimulation of the motor cortex (applied during training) in a skill learning paradigm involving multiple sessions (Reis et al 2009; Fritsch et al 2010): in that setting the influence of stimulation may be ascribed to a physiological effect on cortical function that outlasts the period of stimulation. In the present experiments, however, performance at 2 hours follow-up was relatively depressed even in the L-Dopa alone sessions, suggesting that the subsequent offline improvements took effect only well after blood levels of the medication had declined from their peak: this implies that the beneficial effects of L-Dopa’s interaction with training observed here took more than 2 hours to develop, perhaps occurring overnight (Walker et al 2002), and we would suggest that an effect on protein synthesis seems likely. It is recognised that de novo protein synthesis within the motor cortex is a necessary condition for improvement in a motor skill beyond the first training session (Luft et al 2004) and an effect on this process would seem a reasonable candidate for the influence of L-Dopa on offline changes observed here. A specific enhancement of consolidation by L-Dopa was observed in a spatial memory task in rats, where it was suggested that the medication facilitated relay of the memory to pre-frontal regions for long-term storage (Reinholz et al 2009). Whether in the present study this consolidation process reduces the effects of interference between Days 1 and 2 (Brashers-Krug et al 1996) or encourages overnight

self-rehearsal as observed in sequence learning (Walker et al 2003b) cannot be discerned, as the effect of sleep was not tested here.

6.4.4 Methodological considerations

We employed a task that involves maximising peak thumb acceleration in a given direction, guided by online visual feedback. We chose this task as improvements occur rapidly in the course of a single session, are associated with excitability changes within the primary motor cortex (our stimulation target) and can be accelerated by facilitatory cortical stimulation in the form of TMS or tDCS in healthy subjects (Muellbacher et al 2001; Reis et al 2009; Teo et al 2010). Improving performance in this task relies upon increased selectivity of the motor output and such selectivity is crucial to the performance of fine movements which patients find difficult after stroke. We did not set patient selection criteria too tight with regard to impairment in order that our group would reflect the clinical population at whom training in fine motor tasks may be targeted: our group had a range of impairment from mild to moderate. Although it is possible that patients at one or other end of this scale of impairment may benefit more from such training we did not observe a relationship between baseline clinical scores and improvement within the training session (correlations not significant). The negative relationship observed here between pre-training performance and within-session improvement suggests an unsurprising tendency for performance to approach an asymptote in the course of only one session. The fact that similar negative correlations were observed at later time points, between performance in Block 8 and subsequent changes, on the other hand would suggest that such a within-session ceiling effect does not appear to limit further gains.

Finally, we did not observe the increase in MEP amplitudes after TBS expected in healthy subjects and observed in the patient study of Talelli et al (2007). The likely explanation for this is that MEPs were recorded here immediately after completion of TBS, in order that the peak stimulation effect would coincide with the start of training, and this was probably too early for the excitability change to be observed.

6.4.5 Implications for training strategies

The present results show distinct effects of a single training session under the effects of these 2 interventions on immediate motor performance and on the discrete stages of learning: online changes (early and late), offline changes and the retention of behavioural gains. This provides further evidence to support the notion that these stages are mediated by distinct mechanisms (Karni et al 1998; Luft & Buitrago 2005; Reis et al 2009). We have employed a strategy which combines the non-focal action of a neuromodulator with the focal action of brain stimulation, such that a combined effect is produced within a specific brain region. The result was a synergistic effect on immediate task performance but a relative attenuation of subsequent training effects: thus interventions which enhance performance over a single session do not necessarily enhance learning. For example, it would be important to understand more clearly how reconsolidation occurs after a single training session, as the effects observed here in the course of a single training session may differ when multiple sessions are combined (Dudai & Eisenberg 2004; Censor et al 2010). Either TBS or L-Dopa alone enhanced early training effects without altering the total gains made within the session. A possible implication is that equivalent therapeutic gains could be achieved in shorter sessions using such interventions, but we can only

speculate as to whether the early gains would have been retained without the remainder of the session. This differs from previous experiments described in Chapter 5, where TBS produced an early benefit in training which was maintained to the end of the session, but it should be noted that in that case the training sessions were shorter and in stroke patients fatigue may play more of a role in limiting the total gains achievable within a given session. Thus it is conceivable that with multiple shorter training sessions the early benefit of TBS or L-Dopa may have resulted in accumulated additional gains, further emphasising the importance of the training schedule (Tanaka et al 2010). The offline improvement with L-Dopa is encouraging and in keeping with the reported beneficial effects of this medication on motor memory formation (Floel et al 2005a; Floel et al 2005b) and on clinical recovery following stroke (Scheidtmann et al 2001). The lack of change in our clinical measures suggests that the changes induced by training were specific to the task and/or number of training sessions. On the basis of these experiments, a trial of these interventions over multiple shorter sessions would seem the logical next step. For such a study, a factorial design (rather than cross-over) with larger patient numbers would seem sensible given our present results. More generally, this work emphasises the importance of considering a training schedule as a series of stages and provides encouragement for those seeking to apply rational physiological interventions to improve motor outcome following stroke.

Patient	Medical History	Medication
1	Colonic carcinoma with colectomy, Hysterectomy, LV dysfunction, PAF	Coumadin, Digoxin, Corgard, Cardizem, Fosamax
2	Hypertension, Diabetes	Aspirin, Lisinopril, Simvastatin, Copidogrel, Metformin, Hydrochlorthiazide
3	Transient arrhythmia	Coumadin
4	Dyslipidaemia, Mitral valve prolapse Orthostatic hypotension, Asthma	Fludrocortisone, Niacin
5	Atrial myxoma, Prostatic carcinoma Hypertension, Hypercholesterolaemia	Lisinopril, Hydrochlorthiazide, Atorvastatin, Metoprolol, Citalopram
6	Carotid body tumour, Hypercholesterolaemia	Clonazepam, Venlafaxine, Atorvastatin
7	Intermittent hypertension, Carotid stenosis	None
8	Hypertension, Hypercholesterolaemia Hysterectomy	Atorvastatin, Amlodipine, Hydrochlorthiazide, Aspirin, Dipyridamole
9	Hypertension, Hypercholesterolaemia	Hydrochlorthiazide, Lisinopril, Pravastatin
10	No prior history	Aspirin, Dipyridamole, Atorvastatin
11	Hypercholesterolaemia	Aspirin, Atorvastatin
12	Hypertension, Hypercholesterolaemia Paroxysmal hemicranias, Hypothyroidism	Aspirin, Pizotifen, Sertraline, Ezetimibe, Candesartan, Levothyroxine

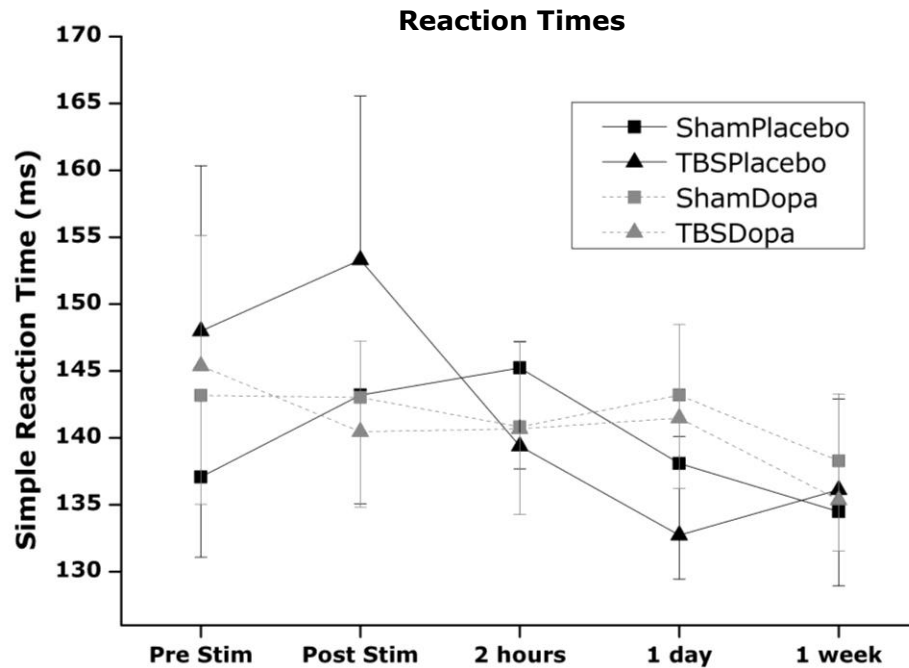
Supplementary Table 6.1. Patient medical history and medications

(LV = Left Ventricular, PAF = Paroxysmal Atrial Fibrillation)

	MEP pre-stimulation (mVms)	MEP pre-training (mVms)
Sham-Placebo	3.76 ± 0.92	3.27 ± 0.69
TBS-Placebo	2.81 ± 0.61	2.59 ± 0.48
Sham-Dopa	3.44 ± 0.67	3.12 ± 0.61
TBS-Dopa	3.43 ± 0.75	3.58 ± 0.88

Supplementary Table 6.2. Mean baseline values for MEP areas

MEP, Motor Evoked Potential; TBS, Theta Burst Stimulation. Mean ± SE. MEP areas did not change immediately following TBS (3-way Drug x Stim x Time ANOVA, No significant interactions or main effects; all $F < 2.6$, $P > 0.130$). In relation to motor training (Pre, Midpoint, Post, 2 hours, Day 2, 1 week) MEP areas were greater following Dopa (main effect of Drug, $F_{1,11} = 7.4$, $P = 0.020$) but no other main effects or interactions were significant (all $F < 1.6$, $P > 0.270$).



Supplementary Figure 6.1. Simple reaction times

Reaction times became shorter in the course of a given session (significant main effect of Time) but did not vary according to session type (no main effect of Intervention or Intervention x Time interaction: see text for ANOVAs).

Chapter 7

Discussion

The key themes of the work presented here are i) the physiological changes in motor control following stroke, ii) the mechanisms underlying motor learning and the potential to enhance this using brain stimulation, and iii) whether these principles may be applied to achieve meaningful improvements in motor recovery following stroke. In discussing these themes we will first identify questions arising from the main experiments presented. We will then address the wider issue of whether it is feasible to produce clinically meaningful gains in patients with stroke using such an approach, and how this may be achieved.

7.1 The evolution of cortical physiological changes after stroke

In Chapter 3 we present detailed physiological studies acquired longitudinally over a 6 month period following first ever ischaemic stroke. In Chapter 4 we used paired coil TMS and concurrent TMS-MRI to examine the how the contralesional dorsal premotor cortex (PMd) interacts with the wider cortical motor network after stroke to support movement of the paretic hand. The longitudinal stroke study employed single- and paired-pulse TMS to acquire measures of corticospinal and intracortical excitability respectively. The key findings were a high degree of early within-patient physiological variability, declining corticospinal excitability in the intact hemisphere with time, bilateral intracortical disinhibition and a dynamic relationship between cortical physiological parameters and clinical function, with strikingly different patterns observed for corticospinal versus intracortical excitability.

We observed significant variability in all physiological parameters over the first 3 weeks when multiple observations were made, which did not relate in any obvious way to clinical status. The reason behind the instability of these measures during this

acute phase is unclear at present. Stroke is frequently complicated early on by metabolic disturbance resulting from factors such as hypoxia, cardiovascular instability, intercurrent infection and the complications of immobility, and one might reasonably expect such systemic factors to affect cortical physiology. However in the absence of longitudinal correlations between physiological parameters and clinical scores across this period this can only be supposed. Such instability is not believed to be present in healthy subjects (Uy et al 2002; Strutton et al 2003) and is therefore presumably pathological. The unfortunate implication of this finding, however, is that in order to obtain reliable physiological measures during this period it is likely to be necessary to make more than one assessment.

Corticospinal excitability was reduced in the affected hemisphere as previously reported: although it recovered to an extent it remained sub-normal at 6 months. Excitability in the unaffected hemisphere declined across the 6 month interval but was never raised in comparison to our healthy control group. While hyper-excitability has been reported previously in the intact hemisphere (Delvaux et al 2003), it cannot therefore be said to have been present in our patients. In attempting to explain this decline one might speculate that the partial recovery of excitability in the affected hemisphere over time could allow transcallosal inhibition of the intact hemisphere to resume. This cannot be substantiated without longitudinal direct measurements of interhemispheric inhibition, not included in this study.

In addition to the previously reported reduction in Short Interval Intracortical Inhibition (SICI) in the affected hemisphere, we further demonstrated that Long Interval Intracortical Inhibition (LICI) was also reduced. Although at a group level

measures of intracortical excitability in the unaffected hemisphere did not differ from those recorded in our matched healthy control subjects, the significant correlations with clinical status suggest the presence of reduced SICI and LICI and increased Intracortical Facilitation (ICF) on the intact side of the brain in more impaired patients. Although these paired pulse parameters are well characterised, and can be related in some cases to specific neuronal circuits, the outcome of such conditioned stimulation paradigms is likely to represent the summed influences of a number of intracortical populations. The striking clinical correlations of clinical impairment with reduced SICI / LICI and increased ICF thus suggest an overall shift from inhibition towards facilitation in more affected patients.

Crucially the clinical correlations with measures of intracortical excitability were dynamic: they were absent in the first 3 weeks (except for LICI in the affected motor cortex), became apparent in 5 intracortical excitability measures at the 3 month time point but disappeared again by 6 months. There was no overall change in disinhibition from the acute period to 3 months, but while there was widespread disinhibition acutely values normalised by 3 months in patients with good recoveries. By contrast, clinical correlations with corticospinal excitability measures became less strong across the 6 month period, though still present. We have interpreted this window of strong paired-pulse excitability correlations at 3 months as suggesting that intracortical disinhibition is crucially relevant to effective motor function at that point. This is analogous to the rapid reduction in GABAergic inhibition observed in response to ischaemic nerve block of the contralateral arm in healthy humans (Ziemann et al 1998; Levy et al 2002). Such disinhibition is likely to underlie the changes in somatotopic cortical maps observed with motor learning (Nudo et al

1996a) and after ischaemic stroke (Nudo & Milliken 1996) in animal models, as a result of synaptic changes in the cortico-cortical horizontal connections that define such maps (Ghosh & Porter 1988a). The relationship of clinical motor function to changes in intracortical excitability in the present study would be compatible with a model in which disinhibition provides access via horizontal connections to remote regions in the face of interruption of the primary corticospinal projection. However, although disinhibition is present in the acute phase the correlations only emerge at 3 months. We suggest that the sudden release of connections to remote regions does not become functionally useful until motor practice and physical therapy have allowed such new networks to become organised, and this change may explain the emergence of such correlations.

The dynamic clinical-pathophysiological relationship described above raises the question of whether the first 3 months may represent a therapeutic window, with enhanced capacity for plasticity in response to therapeutic intervention. One study using TBS in acute stroke has suggested that the capacity for plasticity induction in this phase may predict recovery (Di Lazzaro et al 2010). Clinical experience suggests that the most marked improvement in motor function occurs in this interval, but reasons for this are likely to include the early resolution of infarct-related oedema, metabolic disturbances and intercurrent medical complications. On the other hand, intensive therapy can certainly be effective later on and there are many studies describing effective interventions in the chronic stage. This question may be addressed by formally assessing the response to plasticity induction in the acute versus chronic phases, and relating the results to intracortical excitability.

The relatively small patient group studied does not unfortunately allow for a stratified analysis of the effect of lesion location on physiological changes. There is some evidence that the direct involvement of primary motor cortex or of transcallosal fibres may give rise to different pathophysiological changes from strictly subcortical infarcts (Liepert et al 2005) and it would be useful to know whether the clinical-pathophysiological relationships described for the group as a whole are confined to one sub-group or pertain more generally.

After the early reorganisation described above, a chronic state is arrived at in which disinhibition and movement-related over-activity in the contralesional hemisphere is a widely reported feature. The significance of this contralesional disinhibition is controversial. It is well recognised that patients with hemiparesis after stroke recruit contralesional motor and premotor cortical regions when moving the paretic hand, and that the extent of such activation is greater in more impaired patients. What is less clear is whether this extra-activation and intracortical disinhibition contributes usefully to motor function as part of an adaptive response or rather interferes with motor function, an example of maladaptive plasticity such as that proposed to occur in dystonia (Quartarone et al 2009). The demonstration by Murase and colleagues (2004) of excessive pre-movement interhemispheric inhibition targeting the motor cortex of the stroke hemisphere provides support for the latter scenario and has prompted the therapeutic strategy of down-regulating excitability in the contralesional hemisphere, with some promising results (Fregni et al 2006; Takeuchi et al 2008, Grefkes et al 2010). This concept of detrimental inhibition from the contralesional primary motor cortex has also provided support for the therapeutic techniques of Constraint-induced Movement Therapy, which is associated with clinical gains (Wolf

et al 2006) and expansion of motor maps in the stroke hemisphere (Sawaki et al 2008). The same does not appear to be true for the contralesional PMd however. Unlike the contralesional motor cortex, the PMd adopts the M1-like property of force scaling after stroke (Ward et al 2007) and studies using TMS to disrupt movement suggest a constructive role for this region in the recovered motor system (Johansen-Berg 2002; Lotze et al 2006).

The paired coil TMS and concurrent TMS-MRI study described in Chapter 4 investigated the interaction of the contralesional PMd with the cortical motor network. We found that the interhemispheric influence of the contralesional PMd on the ipsilesional motor cortex at rest, inhibitory in the healthy population, was by contrast facilitatory in more impaired stroke patients and correlated with clinical scores of motor function. We observed a posterior shift in the site of peak motor activation within the ipsilesional primary motor cortex with increasing motor impairment. Using concurrent TMS-MRI we demonstrated greater motor state-related influence of the contralesional PMd on ipsilesional sensorimotor cortex with greater impairment, and on the posterior region of ipsilesional motor cortex with greater derangement of the physiological marker of PMd-M1 influence.

While the PMd-M1 interaction tested in this study is inhibitory in healthy subjects at the stimulus intensities used, facilitation can be demonstrated under different conditions (Baumer et al 2006). As discussed above for paired pulse intracortical excitability measures, it is likely that the overall interaction represents the summed influences of several neuronal populations: the change observed here in more impaired patients is thus likely to reflect a shift from inhibition towards facilitation

similar to that observed within the motor cortices. One cannot distinguish from this result alone whether the abnormality in this interaction originates in the contralesional PMd or alternatively results from abnormal ‘gating’ of this interaction within the target motor cortex (the same criticism arguably applies to interhemispheric inhibition in the Murase study). However the relationship between the state-dependent influence of PMd on M1 and impairment suggests that the abnormality in this interaction has relevance for the control of movement. More information about how this may adversely affect motor control would be gained from using paired coil TMS to test this interaction during movement preparation, as was successfully done by Koch and colleagues in healthy subjects.

This study is encouraging in that it demonstrates the feasibility of applying the novel concurrent TMS-MRI technique developed by Bestmann in patient groups to answer clinical questions. This technique allows the investigator to probe the interactions of a candidate cortical region with any other brain region in the imaging field, using TMS as an input to induce haemodynamic changes in functionally connected regions. Moreover this can be done in relation to a task to identify state-dependent changes in such interactions. Whereas paired coil TMS techniques rely upon an evoked potential to provide a measure of cortico-cortical interactions, limiting the target region to the motor cortex, concurrent TMS-MRI does not have this limitation. The price paid for this wider spatial field, however, is reduced temporal resolution.

Taken together with previous studies, the work presented here suggests that the contralesional PMd plays a positive role in movement of the paretic hand via an interaction with ipsilesional motor cortical regions. This raises the question of

whether this region may represent an effective therapeutic target for brain stimulation, aiming to increase excitability within the contralesional PMd. Of the several studies using non-invasive brain stimulation to down-regulate activity in the contralesional primary motor cortex (summarised in Nowak et al 2009), 2 applied cathodal transcranial direct current stimulation to the unaffected hemisphere (Fregni et al 2005; Boggio et al 2007). Although these demonstrated modest improvements in clinical scores of paretic arm function (12% and 10% respectively) one must question whether collateral inhibition of the contralesional PMd using this non-focal technique may have partially negated the benefits of inhibiting the contralesional M1. More focal facilitatory stimulation targeting the contralesional PMd may represent a promising line of investigation.

7.2 Pharmacological modulation of Theta Burst Stimulation, and its application to motor learning

In Chapter 5 we present experiments describing the effect of intermittent Theta Burst Stimulation (iTBS) delivered to the motor cortex on corticospinal excitability in the presence of 3 medications: nicotine, dextro-amphetamine and levodopa. Following iTBS alone excitability is significantly increased for around 15 minutes before returning to baseline levels. In the presence of nicotine there was a period of facilitation which started later, was greater and more prolonged than with placebo, being present at 40 minutes. In the presence of dextro-amphetamine or levodopa the evolution of excitability following iTBS did not differ from that observed with placebo.

Potential mechanisms by which nicotine may have enhanced the effects of iTBS include the facilitation of LTP induction within the motor cortex (both pre- and post-synaptic mechanisms are described) and the reduction of GABAergic inhibition. The involvement of LTP in the excitability change induced by iTBS is a little controversial. While there is no doubt that modulation of the NMDA receptor affects the outcome of iTBS (Huang et al 2007; Teo et al 2007), the duration of facilitation induced is relatively short when compared to the stable changes demonstrated by LTP induction in the rat hippocampus (Larson et al 1986). A recent theoretical model which accurately explains the effects of continuous versus intermittent TBS invokes competing LTP-like and LTD-like synaptic changes (Huang et al 2011). One way to determine whether modulation of GABA activity may have contributed to the enhanced excitability increase observed with nicotine would have been to test SICL. This parameter does not alter in the presence of nicotine alone, however (Orth et al 2005), and it is conceivable that nicotine affects activity at GABA receptors other than those which mediate this form of inhibition. A better approach may be to test the capacity for Paired Associative Stimulation (PAS), an NMDA-dependent LTP-like plasticity induction paradigm, immediately after iTBS. Regardless of mechanism, the combination of iTBS and nicotine appears promising as a means of inducing a relatively prolonged excitability increase. This result, together with previously reported differences in cortical inhibitory phenomena in chronic smokers (Lang et al 2008), also suggests that subjects who smoke should be excluded from plasticity induction experiments.

The absence of modulation of the effects of iTBS by dextro-amphetamine or levodopa was somewhat surprising, as both noradrenergic and dopaminergic modulation of

behavioural plasticity paradigms are recognised and dopaminergic stimulation has well documented effects in direct current stimulation and PAS. The possible reasons for this result are discussed in Chapter 5. As the focus of this experiment (and the subsequent behavioural experiment) was on the effects of nicotine the other 2 medications were only tested at 1 dose. With particular regard to levodopa, whose dose-response relationship is non-linear across a range of paradigms, this is unlikely to be sufficient and we do not consider this to be definitive evidence of no interaction with the effects of iTBS. Further studies are required across a range of doses in order to clarify this matter.

In Chapter 5 we also present experiments testing the effects of iTBS on the outcome of a ballistic thumb movement learning paradigm, chosen as it induces rapid behavioural changes associated with an increase in excitability of the motor cortex. We found that iTBS delivered to the motor cortex before the start of training accelerated and enhanced the outcome of training in this task. In marked contrast to the effects on cortical excitability, this behavioural effect was absent in the presence of nicotine. The use of iTBS to enhance subsequent motor learning is potentially exciting, as compared to other forms of brain stimulation iTBS is of low intensity (therefore relatively safe) and is quick to deliver (190 seconds), making it readily applicable in a variety of experimental and even clinical situations. This result does not comply with the predictions of a homeostatic model of cortical plasticity, in which one LTP-like process raises the threshold for further similar processes to occur soon afterwards. However, in a study testing the effects of PAS on subsequent motor learning Jung & Ziemann (2009) also found that facilitatory PAS given immediately before training in fact enhanced the outcome (a non-homeostatic interaction), whereas

with an interval of 90 minutes before training the expected attenuation of learning was observed (a homeostatic interaction). Moreover it has been observed by other investigators that such homeostatic rules may not always govern the outcome of motor training (Kuo et al 2008b).

The apparent dissociation between the effects of nicotine in combination with iTBS on corticospinal excitability (iTBS effect enhanced by nicotine) versus motor learning (iTBS effect blocked by nicotine) prompted a search for factors other than excitability that could explain the behavioural effects observed. As reported in Chapter 5, we found that behavioural variability during the training process was modulated by the interventions in a similar manner to the training outcome, with a correlation observed between variability and training-related improvement. A follow-up experiment showed that iTBS also increased the directional dispersion of thumb movements, a measure of variability chosen to be independent of volitional factors and localised in its likely origin to the motor cortex. In a simple computer model of an analogous behavioural task we demonstrated the feasibility of an increase in output error (but not afferent feedback error) leading to improved task acquisition.

The ballistic thumb movement task chosen for study is ‘non-physiological’ in that it does not resemble manual skill learning in daily life from a kinematic perspective. This is necessarily limiting when attempting to extrapolate training effects in this task to meaningful functional gains in a patient setting: one would not expect such effects to generalise, as motor learning transfer is recognised to be small and dependent on the degree of similarity between tasks studied (Schmidt & Young 1987). However the thumb task has the advantage of simplicity, consisting of a single movement centred

around a single joint, and a recognised association with changes in the motor cortex (Muellbacher et al 2001). We use it here as a paradigm for studying the effects of interventions on motor training while recognising that they are likely to be task-specific.

It is clear that not all motor tasks would suit a constructive role for performance variability. Accurate placement of a peg in a hole, for example, depends on the gradual reduction or elimination of error with practice. However improvement in the ballistic thumb abduction task involves identifying the optimal combination of motor units to produce acceleration in the target direction. This may be seen as analogous to a golf novice aiming to improve their swing without the help of a coach: in the absence of a rule-based strategy, initial training may involve varying each swing at random. If by chance one swing yields a better outcome than previous attempts, this should be remembered and used as a baseline until further variation improves performance even more. The process of remembering a movement presumably involves synaptic strengthening and is well documented. By contrast the means by which performance is allowed to change, and the contribution of performance variability to this process, is less well understood. Our results suggest that it is this aspect of training which was enhanced by iTBS in the current experiments. Our computer model described an inverted U-shaped curve relating output variability to training outcome (Figure 5.2.4): in our interpretation, iTBS moved performance further up this curve. The effect of nicotine in blocking the enhancement could in theory have occurred via a reduction in output variability or via a further increase beyond the curve's peak (akin to the effect of higher doses of dopaminergic stimulation in learning paradigms). An increase in signal-to-noise ratio in the motor

cortex, previously reported with cholinergic stimulation, would favour the former scenario but this is purely speculative. In order to understand this process further it would be useful to test variability more directly, to measure excitability within the same experiment, and further to assess the effects of continuous (as well as intermittent) TBS.

7.3 Modulating motor learning in patients with chronic stroke

Having demonstrated that iTBS delivered to the motor cortex can enhance motor learning in healthy subjects we performed a similar experiment in a group of patients with chronic ischaemic stroke (described in Chapter 6). We employed the same thumb abduction task to the paretic hand and tested the effects of iTBS / sham stimulation in the presence of levodopa / placebo. Levodopa was chosen to be included in this experiment on the basis of previous investigations demonstrating enhanced motor plasticity with dopaminergic stimulation in both healthy subjects and patients with stroke. For this experiment we included additional assessments of task performance the following day and after 1 week. The combination of both active interventions resulted in enhanced pre-training performance. With either iTBS or levodopa (but not both) patients achieved more rapid behavioural gains early on in the training session: however these early gains plateaued with no advantage by the end of training, and in fact a worse endpoint with levodopa alone. By contrast levodopa alone appeared to enhance overnight consolidation, with significant offline improvements by the following day. At 1 week's follow-up there was no difference between the interventions.

While either levodopa or iTBS given in isolation accelerated within-session performance gains it appears that a ceiling effect limited further gains. One possible explanation for this is that the task was too arduous for the patient group, with too many repetitions of movements with the paretic thumb, and that fatigue prevented potential gains from being achieved. On the other hand it maybe that either intervention would allow equivalent improvements to be achieved in a shorter training session. We do not know the effect of the 2 interventions on performance variability in this experiment. Such an analysis is likely to be confounded by a high degree of baseline variability after stroke, but this information would be interesting in assessing whether the positive role of variability observed for this task in healthy subjects (see Chapter 5) also applied to our stroke group, and how this was modulated by levodopa.

It is difficult to be certain whether levodopa improved consolidation of performance gains in this experiment. With this medication alone there was certainly an overnight improvement in performance, in contrast to a slight decline with other session types, but this is likely to result partly from worse performance at the end of training (see discussion in Chapter 6). However in the dopamine sessions patients performed better on Day 2 than in other session types. It is likely to be worth specifically examining this question in future experiments. In attempting to explain an effect of dopaminergic stimulation during the consolidation phase it is necessary to invoke processes downstream of the medication itself, such as changes in consolidation-related protein synthesis (Luft et al 2004), since the pharmacokinetic half-life is considerably shorter than the time during which this process is thought to occur. This also raises the question of whether dopaminergic stimulation may perhaps be more effective if given after training, for example before sleep, in order to maximise an effect on

consolidation. In a study of the effects of transcranial direct current stimulation applied during training in a motor skill task, Reis and colleagues (2009) observed a positive effect of anodal stimulation which built up over 5 consecutive days' training. Interestingly, the stimulation effect was seen to act specifically on the overnight consolidation phase. It may be that dopaminergic stimulation given on consecutive days may likewise confer a cumulative benefit. It would be worthwhile addressing these further questions in future studies.

7.4 Concluding remarks

The motivation behind the work that forms this thesis, and the work of countless investigators in this field, is the prospect of achieving meaningful enhancement of stroke recovery. The ideal scenario that one may envisage sees a patient who has suffered an ischaemic stroke passing seamlessly from hyper-acute treatment (thrombolysis to reduce the lesion extent) straight into an acute / sub-acute phase in which the conventional medical care runs alongside a rational combination of physical therapy with targeted interventions to enhance appropriate neuroplasticity. This may involve, for example, a medication and a physiological intervention (eg non-invasive-brain stimulation) timed so as to complement therapy sessions. The choice and use of such interventions may be decided individually according to clinical and physiological variables particular to the patient.

A number of obstacles and uncertainties stand between the current state of the art and the ideal scenario described above. The several areas of uncertainty are detailed over the course of the preceding pages: chief among these are the questions of what is the most effective intervention, whether this can be enhanced pharmacologically and how

this should be delivered in a targeted manner. We would identify the following specific experimental questions to be addressed in this regard as a matter of priority:

1) When testing the effects of physiological or pharmacological interventions on the outcome of motor training it will be essential to link the effects observed to patient variables: clinical state; physiological measures of corticospinal or intracortical excitability; lesion location; the residual motor network being used. 2) The use of brain stimulation techniques (eg Theta Burst Stimulation) in shorter training sessions over several consecutive days should be tested. 3) The question of whether dopaminergic stimulation enhances overnight consolidation of learning should be specifically addressed. 4) Facilitatory focal stimulation of the contralesional dorsal premotor cortex should be tested in stroke patients as an alternative therapeutic target. This list is far from exhaustive, but resolving these questions would represent a significant next step towards the ideal scenario described above.

Although a great deal of research continues worldwide, attempting to address these and other questions, there are some more general issues relating to how this work is conducted which may need to be resolved before significant progress is made.

Putative therapeutic interventions tend to be tested in individual centres in the form of small ‘proof-of-principle’ studies such as that described here in Chapter 6. It is likely that it will be necessary to coordinate such work across several centres in order to recruit the patient numbers necessary to address these questions in a definitive manner. Such a multi-centre approach will require research networks to function more effectively and for consensus to be reached as to the most effective intervention to be trialed. Secondly, studies of this kind are often designed to minimise clinical heterogeneity in order to prevent a treatment effect being obscured by variability.

However, as discussed above, for an intervention to be delivered effectively the relationship of treatment effect to clinical variables will also have to be tested. Another pertinent question is whether the relatively modest behavioural gains demonstrated in such interventional studies can translate to meaningful improvements in quality of life: this is unclear at present. Finally it is likely in our opinion that interventions delivered in combination will prove to be more effective than those given in isolation: study designs will need to allow for this to be tested.

In the first half of this thesis we have presented work detailing the physiological changes occurring in the motor cortices after stroke, relating this to mechanisms of recovery and testing the interaction of the contralesional premotor cortex with the residual motor network. In the second half we have described the pharmacological modulation of theta burst stimulation applied to the motor cortex, demonstrated an enhancement of motor learning by such stimulation, investigated the role of performance variability in this process and examined the effects of brain stimulation (and dopaminergic stimulation) on the time course of motor learning after stroke. In the current chapter we have proposed how the questions raised by this work may be best addressed, with the ultimate goal of enhancing motor recovery from ischaemic stroke. This work is likely to be worth doing. The physical, psychological, economic and social consequences of hemiparesis resulting from stroke demand that the many and rapid advances in all areas of neuroscience be employed to this end.

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